University of Alberta

Analysis of Pond Food Webs in the Whooping Crane Nesting Area, Wood Buffalo National Park

by

Maria Angela Sotiropoulos

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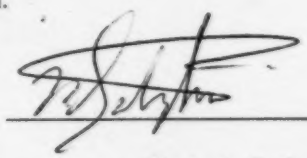

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Analysis of Pond Food Webs in the Whooping Crane Nesting Area, Wood Buffalo National Park** submitted by Maria Angela Sotiropoulos in partial fulfillment of the requirements for the degree of Master of Science in Environmental Biology and Ecology.

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This thesis is dedicated to my parents, Evangelia and Theodore, for their love, support and encouragement.

Σας Αγαπω

ABSTRACT

To determine if the presence of fish influenced the structure of food webs in ponds used as foraging habitats by Whooping Cranes, I sampled aquatic biota and measured environmental characteristics in 36 nesting-area ponds in Wood Buffalo National Park. Principal component analysis indicated that invertebrate communities in ponds with fish were relatively discrete, while fishless ponds were separated into two groups, with beetles and odonates, respectively, occurring as top predators. Isolation from colonization sources and pond morphometry influenced which ponds fish could colonize and persist in, respectively, and which were fishless. Interactions between the biotic and abiotic environment thus contributed to the occurrence of the three community types. Stable isotope analysis revealed differences in the isotopic signatures of food webs containing and lacking fish, with the former being more negative in $\delta^{13}C$ and $\delta^{15}N$, potentially the result of altered carbon sources and different predatory regimes, respectively.

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Chapter I. GENERAL INTRODUCTION

INTRODUCTION

Species can affect each other through many pathways in food webs, and assessing the relative strengths of these various direct and indirect effects is a common challenge to community ecologists (Wootton 1991, Diehl 1992). As predators in aquatic food webs, fish play a major role in regulating ecosystem structure and function (Stein et al. 1995). Many studies focus on the direct impacts that fish have on prey abundances, spatial and/or temporal distributions, and activities (e.g., Allan 1982, Flecker 1984, 1992, Kohler and McPeek 1989). In contrast, little attention has been given to the indirect effects that fish can have on their food webs. Indirect effects can include modifying the distribution and abundance of important resources of invertebrates, such as algae, nutrients, and detritus (Post et al. 1997, Vanni and Layne 1997). Although fish effects have been investigated in a diversity of freshwater habitats, including lakes (Carpenter et al. 1987, Carpenter and Kitchell 1993, Bendell and McNicol 1995), streams (Flecker 1984, 1992, Rosemond et al. 1993, Gilliam et al. 1993), and rivers (Power 1990), food web interactions involving fish in wetlands are relatively poorly understood.

Aquatic invertebrates hold a central role in wetland food webs, serving as a link between primary producers and top predators (Zimmer et al. 2000). Many factors can influence the structure of the aquatic invertebrate community, although the presence of fish may have the greatest potential for altering their abundance and composition (Hanson and Riggs 1995, Zimmer et al. 2000). These fish-induced changes in invertebrate communities can, in turn, influence aquatic bird populations by altering the suitability of wetlands as feeding and nesting areas (Eriksson 1979, Hill et al. 1987, Bouffard and Hanson 1997, Wagner 1997, Cox et al. 1998), ultimately affecting the distribution and abundance of the aquatic birds (Wagner 1997). With this study, I have examined effects of fishes on wetland food webs in Wood Buffalo National Park (WBNP) and how these effects may influence the value of these wetlands to an endangered aquatic bird, the Whooping Crane, *Grus americana*.

An extensive wetland complex in northern WBNP is composed of a mosaic of shallow ponds that are rare and unique in North America (Timoney et al. 1997).

Although comprising typical wetland components, these ecosystems are distinct from other wetlands that occur in the area, having greater amounts of open water and less terrestrial vegetation along their shores. They are commonly associated with reeds, sedges, and bulrushes and contain shallow, clear water virtually devoid of plankton; instead, benthic diatoms dominate the within-pond primary producer community (Timoney et al. 1997). Most of these "diatom ponds" are fed by groundwater and are hydrologically isolated from local streams, although they are subject to spring flooding (McNaughton 1991). Water levels, which likely influence the resident biota, vary both seasonally and among ponds. Internationally significant, this boreal wetland complex serves as the nesting habitat of the last migratory population of Whooping Cranes.

The Whooping Crane is North America's best known endangered species. Standing ca. 1.5 m tall, the whooper acts as an international symbol for the preservation and restoration of all threatened and endangered wildlife. Historically, the Whooping Crane was found throughout much of central and western North America, with breeding grounds extending from central Illinois, north-westward to the Mackenzie River area; wintering grounds were found along the Gulf of Mexico, from Florida to central Mexico (Figure 1-1) (Edwards et al. 1994). Numbers in the late 1800's were estimated at 1500 individuals (Alberta Fish & Wildlife 1991), but as a consequence of hunting, specimen collection, human disturbance, and conversion of nesting habitat to agricultural land, population numbers declined in 1941, until only 16 birds remained (Edwards et al. 1994).

Currently, the breeding population numbers ca. 180 individuals (B. Johns, Canadian Wildlife Service, *pers. comm.*) and is found within a small portion of northern WBNP, with birds over-wintering in and around Aransas National Wildlife Refuge (ANWR), Texas (Figure 1-1). Cranes are sensitive to human disturbance with the majority of deaths, other than chick mortality, occur during spring and fall migration via collisions with power-lines and illegal shooting. The potential of a hurricane or contaminant spill threatens the wintering habitat, which is along a major barge shipping route. Within ANWR, suitable habitat available for range expansion is limiting since this refuge is surrounded by industrial, urban, and recreational development. Similar habitat outside the refuge, although currently not used by the over-wintering cranes, is found east of Houston, Texas, south to Tampico, Mexico (G. Holroyd, Canadian Wildlife Service,

pers. comm.). WBNP is apparently not so limited in that the park is remote and the wetland complex is inaccessible to the general public.

The Canadian Whooping Crane Recovery Team published a revised national recovery plan for the Whooping Crane that calls for the: (1) stabilisation or increase in the existing WBNP population and (2) establishment and support of two other wild migratory populations by 2020 (Edwards et al. 1994). To help achieve these objectives, the recovery plan noted that a greater understanding of the food and habitat requirements of whoopers in WBNP is essential. Information gained would help researchers to identify suitable nesting habitat for potential range expansion within WBNP, as well as aid the recovery team in selecting appropriate nesting areas for re-introductions.

Information on this unique aquatic habitat is deficient because conservation efforts concentrated on monitoring numbers and collecting eggs for a captive-breeding program (Edwards et al. 1994). In response to the revised recovery plan, however, WBNP has recently permitted a limited number of studies to be conducted in this otherwise restricted area. Still, conventional methods of studying food webs, including observational and experimental studies, could not be fully applied to this rare and sensitive pond ecosystem. Stable isotope analysis (SIA) has emerged as a valuable tool for investigating aquatic food webs (Fry 1991) and appeared able to provide a viable alternative for integrating and understanding the structure and function of the pond food webs. In particular, stable isotope ratios of carbon and nitrogen (i.e., $^{13}C/^{12}C$ and $^{15}N/^{14}N$) can be used to obtain descriptions of energy sources and trophic levels, respectively, based on long-term assimilated diets. When combined with the more traditional method of stomach content analysis, which provides a snapshot of that period between ingestion and digestion, SIA becomes a highly valuable approach to food web analyses (Peterson and Fry 1987, Gearing 1991, Vander Zanden et al. 1998, Harvey and Kitchell 2000).

The isotopic composition of an organism is a time-integrated measure of its diet (DeNiro and Epstein 1978, 1981, Fry 1988, Wada et al. 1993, Gannes et al. 1997). Consumers typically have stable carbon and nitrogen isotopic values that are less negative and more positive, respectively, relative to their prey because lighter isotopes (i.e., ^{14}N and ^{12}C) are lost via preferential excretion or respiration and fixation (Macko et

al. 1982, Minagawa and Wada 1984, Keough et al. 1996). Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios exhibit a consistent increase of approximately 0-1‰ and 3-5‰, respectively, with each addition in trophic level (DeNiro and Epstein 1978, 1981, Minagawa and Wada 1984, Peterson and Fry 1987). Differences in diet and subsequent changes in the ratios of heavy to light isotopes of certain elements (i.e., isotope fractionation) lead to distinct isotopic values among organisms that can allow researchers to trace the origin of organic carbon of organisms and identify their trophic positions within a food web (Peterson and Fry 1987, Gannes et al. 1998).

The usefulness of SIA to ecological studies, however, depends on the consistency and suitability of the analytical techniques employed. Much of the error associated with isotopic analysis results from sample preparation alone (Boutton 1991, Kelly 2000), which can, e.g., reduce precision of mass spectrometers and affect interpretation of stable isotope results (Boutton 1991, Griffiths 1991). It is therefore important to test the effects of sample preparation techniques on stable isotope values via comparative laboratory experiments (Bunn et al. 1995, Gannes et al. 1997, Kelly 2000). To date, relatively few such stable isotope studies have been done. Chapter II focuses on the effects of one such technique, lipid extraction, on the stable carbon and nitrogen isotope values of fish tissue and the resulting implications for food-web studies. Because lipid synthesis discriminates against ^{13}C in favour of the lighter isotope ^{12}C, it is common practice to remove the lipid fraction from samples (DeNiro and Epstein 1977, Pinnegar and Polunin 1999). However, the effects that this sample preparation technique may have on other elements being analysed concurrently have not been considered. For SIA to provide successful interpretations in ecological studies, sources of variation must be understood. Therefore, the results obtained from this experiment, as well as those from other studies on preparation techniques, are incorporated into use of stable isotopes in the chapter that follows.

The core of this thesis, Chapters III and IV, focuses on the aquatic food webs of ponds in the Whooping Crane nesting area of WBNP. The study examines the composition and trophic organisation of the pond communities to gain a better understanding of the structure and function of this rare ecosystem. The wetland habitat used by the last migratory population of Whooping Crane for breeding consists of small

shallow ponds that contain relatively abundant, but patchily distributed, fish (Nelson and Paetz 1972, 1974). Preliminary studies had suggested these fish to be key food-web components that link cranes to their habitat (Duxbury and Holroyd 1996, D. Bergeson, Parks Canada, *pers. comm.*). Chapter III focuses on the composition of the invertebrate communities within the diatom ponds, determining the relationships among invertebrates, fish, and the physico-chemical environment via multivariate ordination. In Chapter IV, I use SIA to focus my study on the structure of food webs in ponds with and without fish, the food sources on which fish and cranes rely, and the sources of energy that drive these ecosystems.

By identifying community patterns and trophic pathways, this rare and little-studied boreal wetland can be better understood. The influence of fish on the aquatic invertebrate community within wetlands may be particularly important in understanding the suitability of these wetlands as habitat for other animals that forage in these ponds, specifically the Whooping Crane. Ultimately, this research will contribute information about a virtually unknown aquatic system used by an endangered species during breeding. This will assist the recovery team in understanding patterns of habitat use by cranes in the area, whether appropriate habitat is limiting the expansion of the WBNP population, and what habitat features need to be considered when attempting to establish new migratory populations elsewhere.

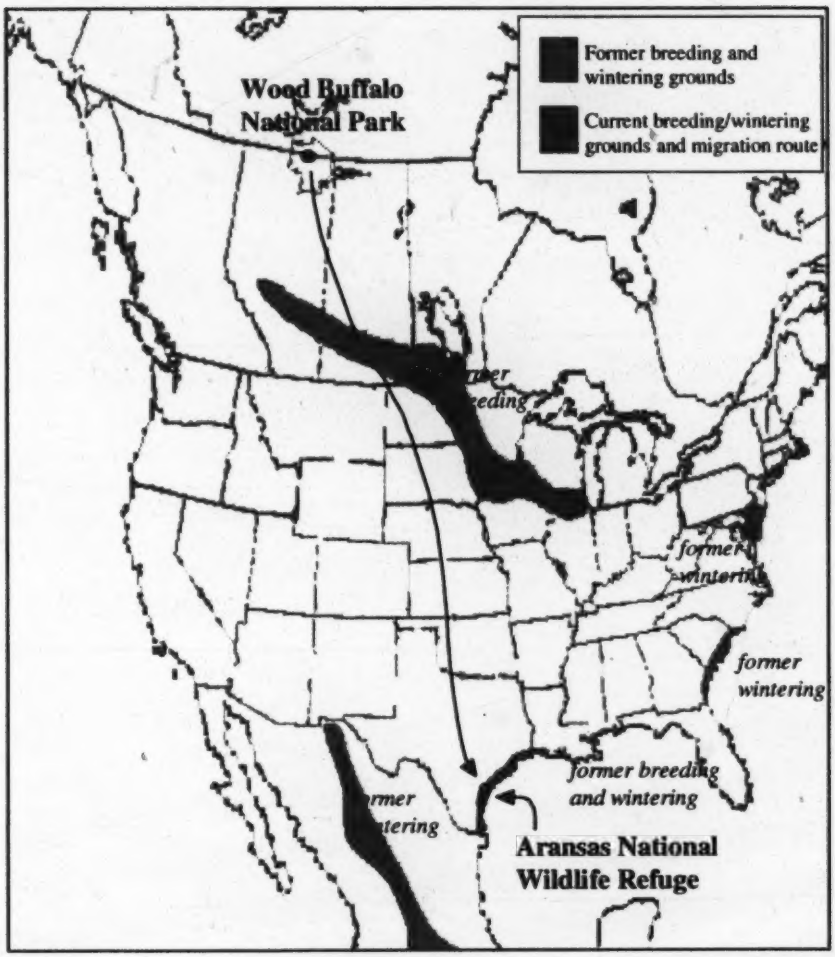

Figure 1-1. Map of North America depicting the historic distribution and current breeding and over-wintering grounds of the Whooping Crane (modified from Meine and Archibald 1996).

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Chapter II. EFFECTS OF LIPID EXTRACTION ON THE STABLE ISOTOPE VALUES ($\delta^{13}C$ AND $\delta^{15}N$) OF FISH TISSUE: IMPLICATIONS FOR FOOD-WEB STUDIES

INTRODUCTION

Stable isotope analysis (SIA) has become an important tool for ecologists to identify energy sources and depict the structure of food webs (e.g., Hobson and Welch 1992, Keough, et al. 1996, Beaudoin et al. 2001). In particular, the stable-carbon isotope ratios of tissues ($^{13}C/^{12}C$) reflect time-integrated sources of carbon to the consumer, while the stable-nitrogen isotope ratio ($^{15}N/^{14}N$) indicates the consumer's relative trophic positioning (Peterson and Fry 1987). However, the efficiency and comparability of biological tissue SIA among ecological studies depends, in part, on the consistency and suitability of the techniques employed by various researchers. Indeed, much of the variability within and among studies using stable isotopic analysis results from non-uniform sample preparation and treatment procedures alone (Boutton 1991, Kelly 2000), which can, e.g., reduce precision of mass spectrometers and affect proper interpretation of stable isotope results (Boutton 1991, Griffiths 1991). It is therefore important to evaluate and test the effects of sample preparation techniques on stable isotope results via comparative and laboratory experiments (Bunn et al. 1995, Gannes et al. 1997, Kelly 2000). Recent studies, for example, have examined the influence of sample preservatives (solvents) and type of sample combustion on stable isotope results (Hobson et al. 1997, Bosley and Wainright 1999).

Another concern arises if sample preparation techniques designed for one particular element (e.g., $\delta^{13}C$) may have undesirable or unanticipated effects on another stable isotope (e.g., $\delta^{15}N$) analysed concurrently using the same sample (Bunn et al. 1995). Modern online continuous-flow isotope-ratio mass spectrometry (CF-IRMS) conveniently measures both carbon and nitrogen isotopes by single small sample combustion and has largely replaced offline means to measure carbon and nitrogen isotopes. The CF-IRMS technique is faster and cost effective, although less precise than labour intensive offline dual-inlet analyses. However, the techniques used to prepare biological samples for online CF-IRMS analysis have typically not changed from offline dual-inlet preparation procedures. A common practice among laboratories conducting

SIA of biological material include acid washing and/or solvent extraction of lipids from samples to remove both extraneous inorganic carbon and to help reduce isotopic variability arising from variable lipid contents (Jackson et al. 1986, Hobson and Welch 1992). Although some studies have investigated the effects of acid washing (Bunn et al. 1995, Bosely and Wainright 1999, Pinnegar and Polunin 1999), few have considered the effects of lipid extraction on multi-element stable isotope analyses.

Lipids are depleted in ^{13}C relative to carbohydrates and proteins (DeNiro and Epstein 1977, Griffiths 1991). Biological tissues frequently vary in their percentage of lipid content and hence lipid enriched tissues (e.g., liver and muscle) generally have more depleted $\delta^{13}C$ values than other tissues that contain little fat, such as hair or nails (Tieszen and Boutton 1989, Pinnegar and Polunin 1999). For this reason lipids are frequently extracted from samples being analysed for $\delta^{13}C$ in order to reduce variability attributed to differences in lipid content among tissues (DeNiro and Epstein 1978, Hobson and Clark 1992b). As such, the analysis provides more specific information on protein or carbohydrate pathways vs. lipid pathways during metabolism (Hobson and Stirling 1997). However, the effects that lipid removal by solvent extraction on the $\delta^{15}N$ values of specific tissues remains unclear. More recently have studies begun to examine this question using liver of broad whitefish (*Coregonus nasus*) (Hesslein et al. 1993) and various tissues of rainbow trout (*Oncorhynchus mykiss)* (Pinnegar and Polunin 1999).

The goal of this study was to determine the effects of lipid extraction procedures on the stable-carbon and -nitrogen isotope ratios of muscle tissue from dace (*Phoxinus* sp.), and brook stickleback (*Culaea inconstans*). I also compared the effects of the standard solvent-based procedures on whole juvenile fish and muscle tissue from adults using laboratory-held fathead minnows (*Pimephales promelas*) fed on a commercial diet.

MATERIALS AND METHODS

Brook stickleback ($\bar{x}$ = 50.8mm;1.32g) and dace ($\bar{x}$ = 69.4mm; 3.29g) were collected from a single pond in Wood Buffalo National Park, NWT, Canada. Fathead minnows (adult $\bar{x}$ = 67.7mm; 3.41g, juvenile $\bar{x}$ = 35.0mm; 0.46g) were collected from a pond near Athabasca, Alberta, Canada. They were maintained on pelleted fish food at the

University of Alberta Aquatics facility for 8 months prior to the sample processing experiment.

Treatment of tissue for adult fish and juveniles varied because the use of whole-bodied juvenile fish required the voiding of guts for subsequent SIA. Immediately after collection, adult brook stickleback and dace were killed and frozen at –20°C until shipped to the University of Alberta for further processing. Fathead minnows were killed and processed immediately. Half the muscle tissue from individual fish was assigned control (no lipid extraction) and the other half was subjected to lipid removal (treatment). Juvenile fathead minnows were held without food for 48h to allow voiding of guts before being killed. Control samples were rinsed with distilled water and air-dried. Treatment samples were cut into small pieces and soaked in a 1:1 chloroform:methanol solution three times, then rinsed with distilled water and air-dried. Once dry, samples were ground to a fine powder using a mortal and pestle. For stable isotope analysis, approximately 1mg of homogenous material was weighed into 4 x 9 mm tin cups. Samples were analysed for stable isotope ratios of carbon and nitrogen using a Micromass Optima™ EA CF-IRMS at the National Water Research Institute, Saskatoon, Saskatchewan, using standard techniques. Isotope ratios are expressed in delta (δ) notation as parts per thousand (per mil, ‰) differences from a standard as follows:

$$\delta^{13}C \text{ or } \delta^{15}N \text{ (‰)} = [(R_{sample} - R_{standard})/R_{standard}] \times 1000,$$

where R denotes $^{13}C/^{12}C$ or $^{15}N/^{14}N$. All results are reported relative to Pee Dee Bee limestone standard (PDB) for $\delta^{13}C$ and to atmospheric nitrogen (AIR) for $\delta^{15}N$. International standards and a working protein standard ($\delta^{13}C$ = -12.6±0.1, $\delta^{15}N$ = 5.6±0.1) was used to determine sample repeatability. Results are expressed as mean ± standard error.

I used paired t-tests to assess differences in $\delta^{13}C$ and $\delta^{15}N$ values among the control and treatment samples of adult muscle within each species and two-sample t-tests for juvenile minnows. A difference was considered significant when $P < 0.05$. To examine effect of lipid removal on sample variance, Bartlett's test of homogeneity was run.

RESULTS

Whole Juvenile Fish

As expected, $\delta^{13}C$ values of whole juvenile fathead minnows treated for the removal of lipids ($\bar{x}$ = -19.0‰, n = 10) were significantly enriched in ^{13}C relative to control samples ($\bar{x}$ = -22.4‰, n = 9) by approximately 3‰ (Figure 2-1). Similarly, $\delta^{15}N$ values of treated whole minnows ($\bar{x}$ = 11.7‰, n = 10) were significantly enriched in ^{15}N relative to control samples ($\bar{x}$ = 8.9‰, n = 9) (Figure 2-2). Sample variance between control and treatment samples did not differ for either carbon (B = 0.11, P = 0.74) or nitrogen (B = 0.31, P = 0.58).

Adult Muscle Tissue

Muscle tissue of lab reared adult fathead minnows ($\bar{x}$ = -24.5‰; n = 9), wild dace ($\bar{x}$ = -29.1‰; n= 10), and wild brook stickleback ($\bar{x}$ = -28.2‰; n = 17) that were treated for the removal of lipids were all enriched in ^{13}C relative to untreated tissues ($\bar{x}$ = -24.9, -30.1, and -29.0‰, respectively) corresponding to a positive shift in $\delta^{13}C$ values of +0.4, +1.0, and +0.8‰, respectively (Figure 2-1). However, the magnitude of the change in $\delta^{13}C$ values for muscle was markedly less than that of whole juvenile fish. As with whole-juvenile minnows, sample variance in $\delta^{13}C$ did not differ between control and treated muscle for fathead minnows (B = 0.03, P = 0.87), dace (B = 0.09, P = 0.77), and stickleback (B = 0.06, P = 0.80). Removal of lipids also resulted in small, but significant, increases in $\delta^{15}N$ values of treated samples relative to control samples for fathead minnow ($\bar{x}$ = 11.1‰ vs 10.5‰), dace ($\bar{x}$ = 7.5‰ vs 7.2 ‰), and brook stickleback ($\bar{x}$ = 7.4‰ vs 7.1‰) (Figure 2-2). Similarly, no significant changes in sample variance were evident (B = 0.09, P = 0.76; B = 1.10, P = 0.30; B = 0.78, P = 0.38, respectively).

DISCUSSION

$\delta^{13}C$ Measurements

Lipid extraction affected the stable-carbon isotope values of whole juvenile fish and the dorsal white muscle tissue of adult fish, although not to the same degree. The magnitude of the $\delta^{13}C$ shift due to lipid extraction was more than three times greater for

whole juvenile fathead minnows compared to adult muscle tissue. Among the latter, the smaller increases in $\delta^{13}C$ between field-caught stickleback and dace (+0.8‰ to +1.0‰) were double those of laboratory-held dietary controlled adult fathead minnows (+0.4‰).

Increases in $\delta^{13}C$ following lipid extraction were not unexpected, since lipids generally have more negative and variable $\delta^{13}C$ values than most tissue (Park and Epstein 1961, DeNiro and Epstein 1977, Tieszen et al. 1983). Indeed, the increases in $\delta^{13}C$ that were observed with lipid extracted muscle tissue of adult dace and brook stickleback were similar to that seen for white muscle from rainbow trout juveniles (~0.8‰) (Pinnegar and Polunin 1999). These increases for lipid extracted samples are larger than the error associated with expected analytical variability (±0.2‰) (Gearing 1991). Nevertheless, this enrichment in ^{13}C should not often have a negative impact on tracing carbon sources through many food chains since specific plant types (e.g., C_3 vs. C_4) often differ in carbon-isotopic values (Boutton 1991, Ehleringer 1991, Kelly 2000). However, trophic level assignments may be affected by an enrichment of 0.8 to 1.0‰ since the literature suggests one trophic transfer may range between +0 and +1.0‰ for $\delta^{13}C$ (DeNiro and Epstein 1978, Fry and Sherr 1984). Trophic positioning, however, is more generally assessed using $\delta^{15}N$ since there is a larger isotopic shift per trophic level compared to $\delta^{13}C$ (Focken and Becker 1998, Kelly 2000).

The lower shift in $\delta^{13}C$ (0.4‰) for adult fathead minnow muscle was similar to the effects on muscle tissue of lake trout (*Salvalinus namaycush*), where $\delta^{13}C$ shifts due to lipid extraction were as low as +0.1 to +0.2‰ (Kling et al. 1992). The lower carbon isotope shift observed in fathead minnows could be related to the composition of the experimental pellets that the fatheads were being fed and hence the lipid content of these lab-raised fish. Unfortunately, the lipid contents of the samples were not quantified. The difference in dietary pellet composition relative to the natural diets of dace and sticklebacks may have affected the lipid content and overall $\delta^{13}C$ of the fish tissues (Gu et al. 1996, Kling et al. 1992).

An increase in $\delta^{13}C$ of +3.3‰, however, as seen in the lipid-extracted whole fish, could have important ecological and sample processing implications. Stable isotope carbon isotope analysis is commonly used in studies of aquatic food webs to determine

nutritional sources for animals i.e., the contribution of different primary producers to the energy flowing to animals in a food web (Peterson and Fry 1987). In this case, an increase in $\delta^{13}C$ by 3.3‰ would have underestimated the contribution of macrophytes and overestimated the contribution of phytoplankton to the nutrition of fish in a Lake Superior food web study (Keough et al. 1996). Alternatively, our findings may suggest that the use of whole organisms may not be suitable due to differences in size of various temporally changing tissues (e.g., liver vs. bone vs. muscle).

$\delta^{15}N$ Measurements

Removal of lipids led to increased $\delta^{15}N$ values in both whole juvenile fish and adult muscle tissue, although the shift in the adult muscle tissue by only +0.3 to +0.5‰ is not likely biologically significant. In contrast, lipid extraction increased $\delta^{15}N$ values of whole fathead minnows by 2.7‰, equivalent to a trophic level (DeNiro and Epstein 1981, Minagawa and Wada 1984).

An increase in $\delta^{15}N$ values following lipid extraction could be due to the incidental solvent leaching of proteins (i.e., amino acids) from the tissue. Methanol and chloroform are not solvents specific to lipids but for polar and non-polar compounds, respectively. They are especially effective for lipid removal in combination because together they extract storage, or non-polar, fats and the mostly polar structural fats. The latter are attached to proteins, therefore, use of methanol could very well be leaching out amino acids at the same time as the structural fats are removed, thereby contributing to the enrichment of ^{15}N (R. Doucett, University of New Brunswick, *pers. comm.*). The different degrees of $\delta^{15}N$ value shifts for muscle vs. whole fish suggest that the effects of different solvents on different tissues should be considered more closely. If a suitable non-polar neutral solvent were used, one may be able to extract most of the storage lipids while minimising effects on the $\delta^{15}N$ values (R. Doucett, University of New Brunswick, *pers. comm.*).

Conclusions

The large $\delta^{13}C$ shift observed in lipid extracted whole fish relative to muscle tissue is likely the result of the removal of ^{13}C-depleted lipids from viscera and various

body tissues such as the liver and fat (Robinson and Mead 1973, Saupe et al. 1989), tissues that are lipid-rich and thus are greatly affected by the lipid extraction preparation procedure (Tieszen et al. 1983, Ehleringer 1991). If analyses could be restricted to specific tissue types, one should keep lipid content comparable, without extraction. Muscle tissue is most commonly used in analysis of stable isotopes of vertebrates as it has both an intermediate turnover rate (Tieszen et al. 1983, Hobson and Clark 1992a) and is less variable than other tissues (Pinnegar and Polunin 1999). It is also unrealistic to use the whole body of large organisms.

On the other hand, restricting analyses to specific tissues may provide biased estimates of the isotopic value of organisms because of tissue-specific isotopic fractionations and turnover rates during the incorporation of diet into tissue (DeNiro and Epstein 1978, Gannes et al. 1998). Furthermore, use of a specific tissue type is often not practical when dealing with a diversity of organisms in a food web or with larval and juvenile stages (Minagawa and Wada 1984, Hesslein et al. 1991, Keough et al. 1996). Under these circumstances, whole organisms or the use of several tissue types are recommended to integrate tissue-specific composition and dynamics (DeNiro and Epstein 1978, Tieszen et al. 1983). When whole fish are used, I recommend that they be eviscerated, decapitated, and skinned, leaving only the muscle tissue and skeletal bone for analysis, to minimise differences with samples of muscle tissue. By extracting lipids from such diverse samples, consistency of results should increase; indeed, this was the original reason why this approach was used for stable carbon isotope analysis, although, I did not observe a decrease in variance between control and treatment samples for either $\delta^{13}C$ or $\delta^{15}N$.

To avoid the effects of lipid extraction on $\delta^{15}N$ values, one could analyse separate aliquots of samples for carbon and nitrogen isotopes, treating only the carbon samples for lipids. Similar suggestions have been made with respect to problems associated with acid washing (Bunn et al. 1995, Bosely and Wainright 1999). This, however, would greatly increase the cost of the analysis and the amount of sample material needed.

An alternative to analysing two separately treated sample aliquots is lipid normalisation. Lipid normalisation uses a statistical rather than methodological technique that is implemented post-hoc rather than prior to analysis. The goal is not to

remove all lipids, but to simply bring all samples to a common level of lipid content. This is common practice with ecotoxicologists (Hebert and Keenleyside 1995) but is only recently becoming of interest to scientists using stable isotopes for food web studies (Kline et al. 1998). There are caveats to this technique, such as diminished precision of data and reduced power of statistical tests, which should be taken into consideration (Hebert and Keenleyside 1995). Further unknown and perhaps widely varying $\delta^{13}C$ values of lipids among samples may have to be considered.

Given our results on lipid removal effects, and those documented for acid washing, it is clear that for stable isotope analysis to be successful in ecological studies, sources of variation must be understood. If ignored, they could result in misleading or incorrect conclusions.

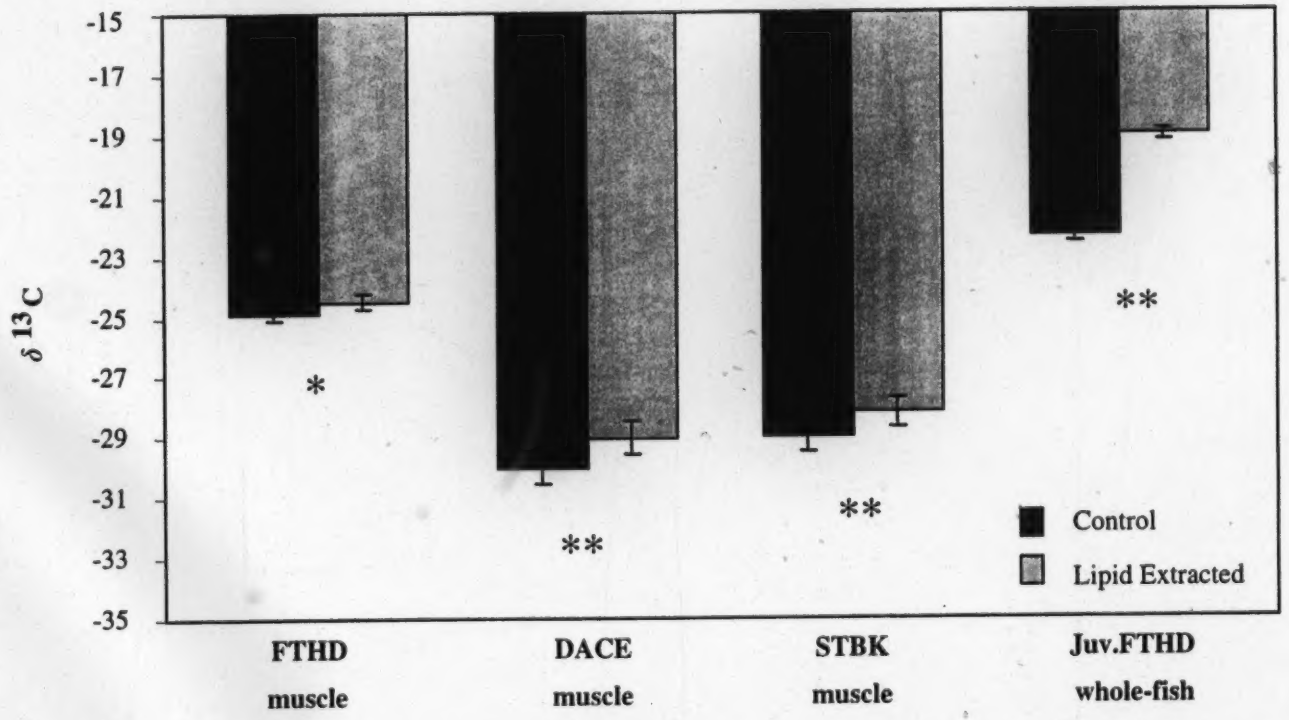

Figure 2-1. Mean (±SE) $\delta^{13}C$ of control and treatment (lipid extracted) samples of adult fathead minnow (FTHD), dace, (DACE), and brook stickleback (STBK) and juvenile FTHD. Significant differences indicated by t-tests (* $P<0.005$; ** $P<0.001$).

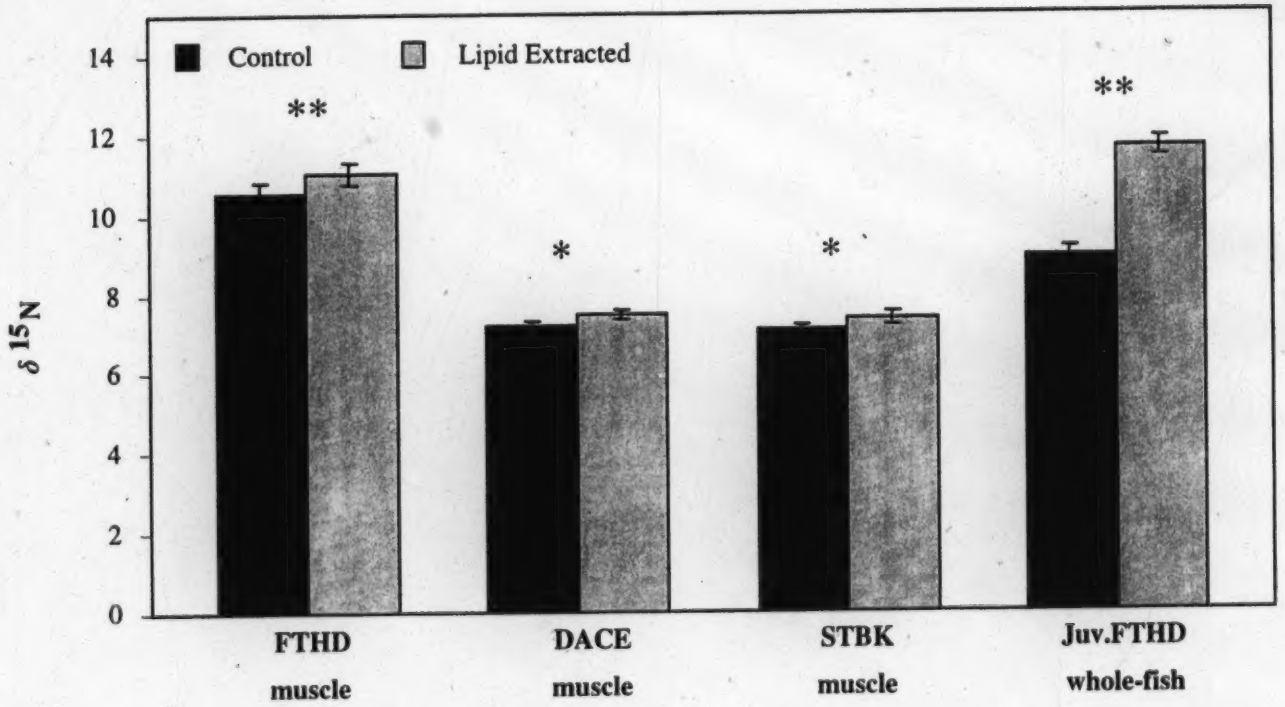

Figure 2-2. Mean (±SE) $\delta^{15}N$ of control and treatment (lipid extracted) samples of adult fathead minnow (FTHD), dace, (DACE), and brook stickleback (STBK) and juvenile FTHD. Significant differences indicated by t-tests (* $P<0.005$; ** $P<0.001$).

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Chapter III. THE ROLE OF BIOTIC AND ABIOTIC FACTORS IN STRUCTURING WETLAND INVERTEBRATES IN WOOD BUFFALO NATIONAL PARK, CANADA

INTRODUCTION

Understanding the factors that shape the structure of communities has long been of interest to aquatic ecologists (Tonn and Magnuson 1982, Bendell and McNicol 1987, Magnuson et al. 1989, Jackson and Harvey 1993, Hanson and Riggs 1995, Paszkowski and Tonn 2000, Zimmer et al. 2001). Community structure is determined by both (a) abiotic effects, such as the physico-chemical environment, that limit the distribution and abundance of species (Tessier and Horwitz 1990, Jackson and Harvey 1993, Schell et al. 2001) and, (b) biotic effects mediated by ecological interactions, principally predation and competition (Gilliam et al. 1989, Power 1990, Hanson and Riggs 1995). Though some studies may focus on the importance of biotic or abiotic factors separately, it is clearly the interaction between both types that ultimately determines the structure of aquatic communities (Robinson and Tonn 1989, McNicol and Wayland 1992, Jackson and Harvey 1993, Bendell and McNicol 1995, Hanson and Riggs 1995, Zimmer et al. 2000).

Aquatic invertebrates hold a central role in wetland food webs, serving as a link between primary producers and top predators (Zimmer et al. 2000). Many factors can influence the structure of the aquatic invertebrate community, although the presence of fish may have the greatest potential for altering their abundance and composition (Hanson and Riggs 1995, Zimmer et al. 2000). These fish-induced changes in the invertebrate community can, in turn, influence aquatic bird populations by altering the suitability of wetlands as feeding and nesting areas (Eriksson 1979, Hill et al. 1987, Bouffard and Hanson 1997, Wagner 1997, Cox et al. 1998), ultimately affecting the distribution and abundance of the aquatic birds (Wagner 1997).

The boreal wetlands of Wood Buffalo National Park (WBNP) provide the feeding and nesting habitat for the last migratory population of Whooping Crane, *Grus americana*. This habitat is composed of a mosaic of shallow ponds that are unique in North America (Timoney et al. 1997). Although comprising typical wetland components, these ecosystems differ from other wetlands in the area by having greater amounts of

open water and less terrestrial vegetation along their shores. They are commonly associated with reeds, sedges, and bulrushes and contain shallow, clear water virtually devoid of plankton; instead, benthic diatoms dominate the within-pond primary producer community (Timoney et al. 1997). The fish fauna of the area is relatively diverse and surprisingly abundant, given the severity of the climate and the small size of the ponds (Nelson and Paetz 1972).

The Canadian and American Whooping Crane Recovery Teams have focused recent conservation efforts on the food and habitat requirements of the whoopers in WBNP, and on understanding the wetland food web that support adult cranes and their chicks (Edwards et al. 1994). Preliminary results of a study initiated by WBNP (D. Bergeson, Parks Canada, *pers. comm.*) suggested that use of ponds by foraging cranes was related to the presence or absence of fish. Therefore, the objective of this study was to investigate the composition of invertebrate communities of the diatom ponds in the Whooping Crane nesting area, determine their relationship with fish and the physico-chemical environment of these ponds, and assess how these relationships may ultimately affect the breeding and survival of Whooping Cranes.

MATERIALS AND METHODS

Description of study site

The study ponds are located in the northern portion of WBNP, Canada, 59°45' - 60°30' N, and 112°45' - 114°00' W (Figure 3-1). This area is located within the Subhumic Mid-boreal ecoclimatic region of Canada, with approximately 50% of the landscape in the core nesting area covered by a mosaic of small ponds (Timoney et al. 1997). Most of the Whooping Crane nesting ponds occur north of 60°N around the Klewi and Sass rivers. Ponds found in Alberta (south of 60°N) are clustered around Preble Creek, a tributary to the Sass River. A few ponds are more isolated (e.g., south of Nyarling River, west of Little Buffalo River) (Figure 3-2).

Area bedrock consists of gypsum karst and it is the dissolution of this gypsum that is thought to strongly influence the water chemistry of the wetland system (McNaughton 1991, Timoney et al. 1997). Most ponds within the wetland are groundwater fed, with water originating from the neighbouring Caribou Mountains, Birch Hills, and Cameron

Hills (McNaughton 1991), and are hydrologically isolated from local rivers and creeks that run through the area (Timoney et al. 1997). Water levels fluctuate seasonally within the wetland. Snowmelt causes spring flooding when ponds, otherwise isolated, are temporarily connected with other ponds, rivers, and creeks (D. Bergeson, Parks Canada, *pers. comm.*), facilitating colonisation of ponds by the biota. Throughout the summer, water levels drop and shallow ponds can dry up by August.

As noted, the 'diatom ponds' in this landscape have greater amounts of open water and less terrestrial vegetation than other wetlands in the area (Timoney et al. 1997). Aquatic plants commonly associated with these ponds include emergent macrophytes, such as bulrush (*Scirpus* sp.) and sedge (*Carex* sp.), submerged plants, such as bladderwort (*Utricularia* sp.) and pondweed (*Potamogeton filiformis*), and the macroscopic alga, *Chara* sp. Surrounding shoreline vegetation includes dwarf birch (*Betula glandulosa*), willow (*Salix* sp.), labrador tea (*Ledum groenlandicum*), marsh cinquefoil (*Potentilla palustris*), and northern reed grass (*Calamagrostis* sp.). Upland forest, which covers less than 5% of the area, consists of narrow ridges and islands of white spruce (*Picea glauca*), black spruce (*P. mariana*), jack pine (*Pinus banksiana*), aspen (*Populus tremuloides*), and tamarak (*Larix laricina*) (Timoney et al. 1997).

Sedimentary peat or diatomaceous earth underlying the ponds is derived primarily from diatoms and other algae, along with some higher aquatic plants (Timoney et al. 1997, *pers. obsv.*). Predominant primary producers in the ponds are benthic diatoms, specifically those associated with high conductivity and alkaline conditions (Timoney et al. 1997, M. Sotiropoulos and M. Agbeti, unpublished data). Diatoms give these ponds their characteristic coloration when viewed from the air. As the ponds dry, the substrate changes from a yellow/pink (when water levels are at the surface) to a crème colour due to the drying of diatoms and the development of a sulphate crust that coats the surface (Timoney et al. 1997).

Field & Laboratory Methodology

From June to August of 1998 and 1999, I sampled 36 ponds. Because of logistic limitations, ponds were only sampled once, with the exception of one that was sampled once each year. Pond selection was based on use by cranes; within each of 18 nesting

areas, one pond was chosen where cranes were observed foraging during a previous observation flight and one was a randomly selected pond (further explained in Chapter IV).

Because nesting-area ponds were sampled only once over two 3-month summer periods, I also sampled five accessible but otherwise similar ponds outside the nesting area of the cranes on a monthly basis in 1999 to evaluate temporal variability in this system. Three of these 'temporal ponds' were located where Preble Creek crosses Highway 5 (PC) and 2 were found ca. 10 km further east (WC) (Figure 3-2).

a) Sampling of Aquatic Biota and Environmental Variables

Aquatic taxa representing all trophic levels were sampled from each pond. Fish were caught in minnow and activity traps (Murkin et al. 1983) that were set for 1-2 hours; overnight sampling (see Chapter IV) indicated that these shorter sets were sufficient to determine fish presence and species composition. Captured fish were identified, counted, and a subset from several size-classes was sacrificed and frozen for further processing.

Aquatic macro-invertebrates were collected from each pond using overnight sets of minnow and activity traps, and by hand with pond nets. Macro-invertebrates were counted and identified to broad taxonomic groups in the field. Benthic fauna were sampled at three sites within each pond from 500-ml cores of the surface sediment. A subsample of invertebrates was brought back to the laboratory alive, separated from sediment, vegetation and detritus, sorted, and frozen for further processing. Zooplankton samples were collected from three sites within each pond using a 243-µm tow net that was hauled horizontally through the shallow water column. Pooled samples were examined under a dissecting microscope and sorted by hand into Cladocera, Copepoda, and Ostracoda.

Water samples were collected from the centre of each pond. Within 2 h of collection, triplicate samples were filtered through Whatman GF/C filters, which were then frozen until shipment for chlorophyll *a* analyses. Total phosphorus samples were treated with potassium persulfate and kept refrigerated until shipment. Samples were sent to the University of Alberta – Meanook Biological Research Station for analysis within 10 days of collection. In the nesting area ponds, conductivity and temperature

were collected on site using a YSI hand held meter while pH was measured using an Orion meter. In the 'temporal ponds', conductivity only was measured in July. Depth was measured at the centre of each pond; thus, measurements taken at the 'temporal ponds' during each sampling period provided information on changing water levels within the wetland system. Other morphometric (area, perimeter) and landscape (distance to major river/creek) characteristics of ponds were measured from maps and aerial photos.

b) Diet Analysis

For the analysis of fish diets, the complete digestive tract was removed from each fish, and contents were sorted and identified to the lowest possible taxonomic level. A standard body part of prey items resistant to mastication and digestion was measured to convert size to biomass, based on published relationships (Smock 1980, Litvak and Hansell 1990) (Appendix A). The frequency of occurrence, and the mean percentage composition by number and by mass of all prey taxa were calculated for each fish species to estimate the importance of prey in a species' diet (Hyslop 1980). These three parameters each contain their own biases that limit the usefulness of any one (Windell 1971, Hyslop 1980), thus I used the relative importance index, RI (George and Hadley 1979), which combines the three. For a given fish species, the RI of prey taxon *i* is calculated as:

$$RI_i = 100\ AI_i / \sum_{i=1}^{n} AI_i$$

where AI_i = the absolute importance of the prey taxon *i*,

= % frequency of occurrence + % total numbers + % total mass;

n = the number of different food types;

% frequency of occurrence = the percentage of all non-empty stomachs containing prey taxon *i*;

% numbers = the percentage that prey taxon *i* contributed to the total number

of prey items in all stomachs;

% total mass = the percentage that prey taxon *i* contributed to the total mass of food in all stomachs.

Detritus was considered separately from invertebrate food items because direct enumeration is not possible. For this food category, the percent frequency occurrence and the mean contribution (volume %) to the stomach contents were averaged and then multiplied by 100 to get a modified index of relative importance.

Food relationships among fish species were analysed using the pair-wise overlap index of Schoener (1974):

$$\alpha_{xy} = 1 - 0.5 \left(\sum_{i=1}^{n} |p_{xi} - p_{yi}| \right)$$

where α_{xy} = the diet overlap between species x and species y;

p_{xi} = the proportion (based on RI) of prey taxon *i* in the diet of species x;

p_{yi} = the proportion (based on RI) of prey taxon *i* in the diet of species y;

n = the total number of prey taxa.

The index ranges from 0 (no overlap) to 1 (complete overlap); Keast (1978) suggested that $\alpha \leq 0.3$ indicates little overlap while $\alpha \geq 0.7$ indicates a high degree of overlap.

Statistical Analysis

Multivariate ordination was used to examine the composition of invertebrate assemblages in ponds, as well as to examine relationships between these assemblages and biotic and abiotic characteristics of ponds. Ordination operates by constructing a series of orthogonal axes from a multivariate data set (e.g., the presence and absence of taxa in ponds) that result in sites being located in positions that (more or less) faithfully portray

among-site similarities in community composition, which can then be related to environmental gradients (ter Braak and Verdonschot 1995, Legendre and Legendre 1998). Indirect ordination (e.g., principal components analysis; PCA) uses only the community composition data to construct axes and position sites; thus axes represent only hypothetical environmental gradients and species-environment relationships must be analysed separately (Jongman et al. 1995, ter Braak and Verdonschot 1995, Legendre and Legendre 1998).

PCA ordinations of biotic and abiotic variables were performed using PC-ORD 4.0 for Windows (McCune and Mefford 1999). A linear response model was chosen based on the gradient length of the first axis following detrended correspondence analysis (ter Braak and Prentice 1988). Presence/absence data were used instead of abundance data in biotic ordinations to reduce potential problems in abundance estimates caused by sampling at different times of the summer. As well, the quantitative biological data collected in the field were categorised only into broad taxonomic groupings, too broad to detect any trends in the data set (Legendre and Legendre 1998; M. Sotiropoulos, unpublished). Ordination analyses were centred and standardised using the correlation cross-products matrix (Greig-Smith 1983). Environmental variables (except pH) were transformed to log(n+1) prior to analysis to homogenise variances and prevent high values and different measurement scales from excessively influencing results (ter Braak and Verdonschot 1995). Relationships between biotic (invertebrate community) and abiotic (environment) data can not be assessed directly with indirect ordination, therefore joint plots were developed using ordination results of specific invertebrate taxa and environmental data (ter Braak 1995).

A Procrustean approach, PROTEST (Jackson 1995), was used to assess the degree of concordance between the invertebrate communities and the pond environments. PROTEST is a permutation procedure used to test matrix concordance based on Procrustean matrix rotation (Jackson and Harvey 1993, Jackson 1995) and has recently been shown to have more power than the Mantel test (Peres-Neto and Jackson 2001). Comparisons were made using pond scores of the first three axes from PCAs of invertebrate and environmental variables. A Monte Carlo randomisation procedure (9999 permutations) was used to evaluate the statistical significance of the analysis.

Multi-response permutation procedure (MRPP), a non-parametric test analogous to discriminant analysis (McCune and Mefford 1999), was performed, using Euclidean distance, to determine if multivariate differences existed between groups of ponds identified *a priori* from PCA analysis.

Finally, to assess the strength of associations between the presence or absence of individual invertebrate species and community types, an indicator species analysis was performed on presence/absence data for each taxon (Dufrêne and Legendre 1997). Significance of the maximum indicator value of each invertebrate taxon was assessed with a Monte Carlo randomisation method, using 9999 permutations (McCune and Mefford 1999). Differences between environmental variables and patterns of community composition were assessed using two-sample t-tests on fish vs. fishless ponds, or on groups identified *a priori* from PCA analysis. A Mann-Whitney test was used to determine if differences observed in invertebrate community composition among fishless ponds were due to sampling dates and not a biological factor. Dates were converted to the Julian calendar. A difference was considered marginally significant at $P < 0.10$ and significant when $P < 0.05$.

RESULTS

Nesting Area Ponds – General

Of the 36 ponds sampled, half contained fish. Ponds containing fish were larger and deeper than fishless ponds (Table 3-1). Total phosphorus levels indicate that the ponds within the nesting area are meso-eutrophic to eutrophic (Wetzel 1983). Chlorophyll *a* samples were taken but were highly correlated with total phosphorus and therefore not used in ordination analyses. Conductivity ranged from 630 µS/cm – 5620 µS/cm, with fishless ponds having the largest range and most extreme values (Table 3-1). Fishless ponds were significantly further from a colonisation source (river or creek) than ponds containing fish ($P<0.03$) (Table 3-1).

Fish species present in these ponds included fathead minnow (*Pimephales promelas*; FTHD), brook stickleback (*Culaea inconstans*; STBK), pearl dace (*Margariscus margarita*), and dace (*Phoxinus* sp.; DACE). Stickleback and DACE were

the most common fishes, each occurring in 94% of the ponds containing fish, whereas fatheads were found in only 22% of these sites.

Invertebrates counted in the field were identified only to broad taxonomic groups (orders, suborders, and families). At this level, composition of the invertebrate assemblages was broadly similar between ponds with and without fish, although Lymnaeidae, Corixidae, and Coleoptera were more abundant in fishless ponds than in ponds containing fish ($P<0.10$) (Figure 3-3). From subsamples brought back to the laboratory, 49 invertebrate taxa were identified; however, after rare taxa (found ≤ 5 ponds) were omitted or combined into larger taxonomic categories to decrease noise (Gauch 1982), only 23 were included in ordination analyses (Appendix B).

Invertebrate Community – Environment Relationships

The PCA of the invertebrate community summarised 28.4% of the total variance in the first two axes (Table 3-2). Ponds containing fish overlapped little with fishless ponds, especially on PC2 (Figure 3-4A). Invertebrate species composition differed between fish and fishless ponds (MRPP: $P=0.002$). Indicator species analysis identified Cladocera and *Graphoderus* sp. as significant indictors of fishless ponds ($P < 0.05$) (Figure 3-4A). Environmental characteristics of fish versus fishless ponds differed in multivariate space (MPRR: $P = 0.014$), as well as for selected environmental variables (Table 3-1). Indeed, PCA of environmental variables revealed similar groupings of fish and fishless ponds as did the invertebrate ordination, with the first two axes accounting for 52.5% of the variance (Table 3-2; Figure 3-4A, 3-5). As a result, the two ordinations were strongly concordant (PROTEST; $P = 0.0007$).

Although differing biotically and abiotically from ponds with fish, the PCA revealed that fishless ponds were not a homogeneous group, but could be subdivided into two groups along PC1 (Figure 3-4A). Fishless ponds with negative scores on PC1 will be referred to subsequently as FL1 and ponds with positive scores as FL2. The two fishless groups differed in composition (MRPP: $P < 0.0001$). Indicator species analysis identified *Graphoderus* sp. as an indicator of FL1 ponds ($P < 0.05$) and Ostracoda, *Caenis* sp., *Aeshna* sp., and *Leucorrhinia* sp. as indicators of FL2 ponds. Differences in invertebrate species composition between the two fishless ponds were not related to the date the ponds

were sampled (Mann-Whitney test: P>0.40). Differences in environmental characteristics between these two fishless groups were also significant (MPRR: P = 0.0001) (Figure 3-4B). Conductivity was greater in FL1 ponds (t-test, P = 0.003), whereas total phosphorus concentrations were marginally greater in FL2 ponds (P = 0.097) (Table 3-1; Figure 3-4B).

Temporal Changes

The five 'temporal ponds' were environmentally similar to the nesting area ponds, although not all variables were measured at these sites (Table 3-3). These 'temporal ponds' were all fishless except for PC6, where a few stickleback and DACE were captured in August. Invertebrate communities collected in these ponds were also similar in taxonomic composition to those of the nesting area ponds.

The PCA of invertebrate communities in the 'temporal ponds' summarised 46.9% of the total variance in the first two axes (Table 3-2). Axis 1 clearly defined the temporal change in invertebrate communities from June to August, with Corixidae, *Lethocercus* sp., and *Caenis* sp. found consistently in later sampling periods, while Coleoptera and Gerridae were found earlier in the season (Figure 3-6). Interestingly PC6 (August), the single pond-month in which fish were present, was an outlier when compared to other ponds sampled in that month. The joint plot of invertebrate communities and environmental variables revealed that total phosphorus and depth were negatively and positively related, respectively, to axis 1 (Figure 3-6).

The nesting area pond that was sampled in both 1998 (Pond 3A-98) and 1999 (Pond 3A-99) varied in invertebrate species composition due to the addition of Copepoda, Anisoptera (*Leucorrhinia* sp. and *Libellula* sp.), and Coleoptera (*Acilius* sp. and Colymbetinae) and the absence of *Caenis* sp. and Chironomidae during the 1999 sampling period. These changes were evident from the positive shift in position along Axis2 (Figure 3-4). Environmental characteristics varied between years as well, with depth, pH, and conductivity increasing from 1998 to 1999, while total phosphorus decreased (Table 3-3).

Diets of Fishes – Trophic Relations

Brook stickleback (STBK)

Thirty fish were analysed for stomach contents, of which one was empty. Ten prey taxa, plus detritus, were found in the stomachs of STBK. Ephemeroptera (33.7% Relative Importance; RI), Chironomidae (21.3%) and zooplankton (i.e., Cladocera, Ostracoda, and Copepoda) (33.9%) were the dominant prey found in the stomachs (Figure 3-7).

DACE

Of the 30 stomachs examined, 7 were empty. The diet of DACE included 13 invertebrate taxa and detritus. Dominant prey included Ephemeroptera (40.2% RI), Chironomidae (12.6%) and detritus (45.5%) (Figure 3-7).

Fathead minnows (FTHD)

Only 4 FTHD's were captured during the short sets, of which one stomach was empty. The prey that were collected from the limited stomachs included Chironomidae (52.1% RI), Ephemeroptera (40.2%), and detritus (46.6%; Figure 3-7).

Trophic Relations

Diet overlap within the fish community in this wetland complex was moderate (0.3 - 0.7) and similar among all taxa; STBK-DACE = 0.612, STBK-FTHD = 0.616, and DACE-FTHD = 0.613, although overlap values with FTHD were based on few stomachs.

DISCUSSION

Community Patterns

Aquatic invertebrate communities in this pond complex are strongly related to the presence or absence of fish. Ponds containing fish had lower abundances of several invertebrate taxa, including Corixidae, Notonectidae, and Coleoptera. Likely a result of predation or competition, reductions in the abundance of such nektonic taxa in water-bodies containing fish have been reported previously (Bendell and McNicol 1987,

McNicol and Wayland 1992, Hanson and Riggs 1995, Zimmer et al. 2000), indicating fish have a major impact on the abundance of nekton within ponds.

PCA indicated that invertebrate communities in wetlands with fish were relatively discrete while fishless ponds were separated into two groups. Coleoptera, Crustacea, and Anisoptera were the three main groups associated with fishless ponds. Crustacea, such as Cladocera, Copepoda, and Ostracoda, are known food of small-bodied fishes (Held and Peterka 1974, Naud and Magnan 1988, Price et al. 1991), therefore, their consistent absence in ponds containing fish is likely a result of predation pressures excluding or limiting their presence (Brooks and Dodson 1965, Hanson and Riggs 1995, Zimmer et al. 2001). Indeed, stomach content analyses revealed that zooplankton made up an important component of the fishes' diet in this wetland system.

Similarly, Anisoptera, such as *Leucorrhinia* sp., a relatively large and mobile dragonfly, are quite vulnerable to fish predation (Nilsson 1981, Morin 1984, Henrikson 1988, Bendell and McNicol 1995), and were consistently absent from ponds with fish.

Aquatic Coleoptera are predators and therefore may be excluded from ponds with fish due to the lower abundance of shared prey items (Bay 1974). However, beetle remains were also found in the stomachs of some fish, and therefore beetles did not completely escape the direct pressures of fish predation (Hanson and Riggs 1995, Fairchild et al. 2000, Zimmer et al. 2000). Although fishless ponds were more isolated from the more temporally stable river and creek habitats, Coleoptera are rapid colonisers (Eyre et al. 1992, Jeffries 1994), thus interactions with fish are implicated in altering the distribution of Coleoptera.

In fishless ponds, invertebrates take over as top predators, with the beetles, *Graphoderus* sp., *Acilius* sp., and the group Colymbetinae consistently found in one set of fishless ponds, while a second, more species-rich community was dominated by Odonata (*Libellula* sp., *Aeshna* sp., *Leucorrhinia* sp., and *Enallagma* sp.). Perhaps the predatory or competitive pressures of beetles on the other invertebrate taxa are strong enough to limit the number of species coexisting with them in these fishless ponds, whereas coexistence with odonates is possible. Within the wetland system of WBNP, dominant pressures of predation are placed on the invertebrate community by fish (when present), which, in turn, prevent or limit the presence and abundance of invertebrate

predators and other taxa (e.g., zooplankton). Within fishless ponds, a dichotomy in predatory guilds occurs, with beetles and odonates alternating as top predator and subsequently affecting the other invertebrate taxa found within the pond community. Thus, biotic interactions (e.g., competition and predation) are suggested to play key roles in structuring the invertebrate community within these ponds.

Community-Environment Relationships

Although the community-level patterns of aquatic invertebrates point to the importance of biotic interactions, particularly predation by fish, ordination of environmental variables revealed significant relations between the presence of fish and the environment, which would indirectly affect invertebrates. PCA scores of environmental variables show fish to be found in larger, deeper ponds. The importance of pond morphometry, particularly depth and area, to fish communities is well recognised (Johnson et al. 1977, Harvey 1978, 1981, Tonn and Magnuson 1982, Robinson and Tonn 1989, Jackson and Harvey 1993). As well, ponds with fish were consistently found closer to one of the rivers or creeks (i.e., stable sources of species for colonisation), supporting the notion that dispersal during spring flooding is critical for fish colonising these isolated ponds (Tonn and Magnuson 1982, Magnuson et al. 1989, McNicol and Wayland 1992, Willis and Magnuson 2000). Thus, whether or not fish occur within these ponds is likely due to the biogeographic conditions of the area (e.g., colonisation), the environmental gradients, or a combination of these and other undefined pressures (Rahel 1984, Magnuson et al. 1989, Tonn 1990, Willis and Magnuson 2000).

The two groups of fishless ponds, characterised by differences in invertebrate community composition, also differed with respect to water chemistry. Specific conductance, although higher in FL1 ponds, was quite high at all sites, which is not atypical for a wetland system, as in WBNP, that is fed by groundwater (LaBaugh et al. 1998, Winter 1999, Mitsch and Gosselink 2000). The groundwater feeding these ponds has a high conductivity resulting from high concentrations of dissolved materials, originating from the bedrock of gypsum karst (Timoney et al. 1997). Higher conductivity was typical of ponds with greater area, similar to other findings (Paszkowski and Tonn

2000), although the exclusion of taxa based on conductivity alone can not be resolved here.

Total phosphorus concentrations also varied between the two fishless groups with higher total phosphorus values observed in FL2 ponds that consistently supporting more invertebrate taxa, suggesting productivity within these ponds may be enhancing the habitat in a way that supports a more diverse invertebrate community.

Temporal Community Patterns and Community - Environmental Relationships

The dynamic nature of food web interactions has been recognised in past studies (e.g., Schoenly and Cohen 1991, Polis et al. 1996). Temporal variation in food web structure can be influenced by many factors, including habitat disturbance and subsequent ecological succession, life history patterns, and predator-prey interactions (Winemiller 1996, Batzer 1998). Although generally similar to those found in the nesting area of the Whooping Crane, the composition of invertebrate assemblages in the five 'temporal ponds' changed from June to August, coincident with temporal variation in environmental conditions. A decrease in depth among all 5 ponds as summer progressed was not surprising given their reliance on limited precipitation following spring floods. Because water permanence is a critical factor for determining the presence of various invertebrate taxa, the drying out of ponds as summer progresses can be reflected in a changing invertebrate community (Schell et al. 2001). Certain taxa may be dependent on particular elements (e.g., for feeding or reproduction) that are not present at all times within the aquatic food web. The ability for biota to adapt to these changes, via various life history traits or them being highly mobile and able to emigrate to nearby ponds, is critical for their existence. The increased levels of total phosphorus may be associated with the decreased water levels in the ponds concentrating nutrients as summer progressed.

Within the 'temporal ponds', beetles and waterstriders, highly mobile predators (Eyre et al. 1992, Jeffries 1994, Fairchild et al. 2000, Spence 2000), were among the dominant taxa early in the summer. As the summer progressed, the community assemblage changed to a more diverse group of invertebrates, which may be associated with the greater availability of nutrients (i.e., increased levels of total phosphorus).

Odonata established themselves later in the season, along with other nektonic invertebrates, when beetles were consistently absent from the ponds, supporting the results found within the nesting area where species rich - Odonata dominated community differed discretely from the species poor - beetle dominated community. Although, differences in the invertebrate community within the nesting area ponds were not a consequence of taxon-specific rates of dispersal, based on sampling dates, but some other limitation (i.e., food source, predation, competition, abiotic environment), that cannot be resolved here.

Interestingly, the fish found in the outlier 'temporal pond' seem to be driving the invertebrate community back to an earlier, less diverse, stage. This pattern subsequently suggesting that predation by fish is excluding certain invertebrate taxa from the aquatic community.

Within the one nesting area pond sampled once in early July and once in early August (albeit in different years), fish were found in both years making comparisons with 'temporal ponds' difficult. The consistent absence of beetles from these ponds likely reflected their exclusion by fish, as discussed earlier. Changes in the abiotic characteristics of the pond were similar to those observed within the 'temporal ponds', specifically the decrease in water depth. Overall, the absence of fish clearly limits the ability of the 'temporal ponds' to act as surrogates for temporal changes that could occur within the nesting area of the Whooping Crane.

Conclusions

Overall, I suggest that fish play a predominate role in structuring the invertebrate community within these ponds. Spring flooding and pond morphometry influence which ponds fish can colonise and persist, respectively, and which ponds will be fishless. The structuring of three distinct pond communities within this wetland system is likely due to the complex interactions between the biotic and abiotic environment (Figure 3-8).

Because the effects of fish lead to distinctly different invertebrate communities, their pressure may alter the suitability of these ponds as foraging sites for aquatic birds (Hurlbert et al. 1986, Spencer et al. 1991, Haemig 1992, Hanson and Riggs 1995, Paszkowski and Tonn 2000), specifically the Whooping Crane. The type and intensity of

the relationship between fish and Whooping Crane in these ponds should depend, in part, on the extent of diet overlap and on the availability of required foods (Hanson and Riggs 1995). Productivity of water-bird populations not only may be strongly affected by fish predation that alters or limits the available food within the system, but also by seasonal trends in invertebrate populations (Hanson and Riggs 1995), both of which were seen within the ponds.

With information gained by this study, along with the stable isotope results that follow (Chapter IV), crane biologists and habitat managers can better understand the link between fish-invertebrate community habitat and the diet of the Whooping Crane. This will ultimately allow scientists to assess 1) habitat use by cranes, 2) whether appropriate habitat is limiting the expansion of the WBNP population, and 3) what habitat features need to be considered when attempting to re-introduce this species elsewhere.

Table 3-1. Means and ranges for environmental characteristics of 36 ponds in the Whooping Crane nesting area. Ponds are placed into three groups (Fish, Fishless 1, Fishless 2) based on the composition of their invertebrate communities. Asterisks denote significant differences by two-sample t-tests (Fish vs. Fishless: *P<0.10, **P<0.05; Fishless 1 vs. Fishless 2: ***P<0.10; ****P<0.005).

Variables	FISH (n=18)		FISHLESS 1 (n=9)		FISHLESS 2 (n=9)	
	Mean	Range	Mean	Range	Mean	Range
Morphometric variables						
Area (ha) *	1.46	0.02 - 7.10	1.05	0.03 - 4.70	0.19	0.02 - 0.63
Depth (cm) *	29.9	16 - 61	24	11 - 35	23	14 - 38
Chemical Variables						
Conductivity (uS cm^{-1})****	2353	1266 - 3683	3018	846 - 5620	1487	630 - 2200
pH **	7.47	6.62 - 8.40	7.9	7.3 - 8.4	7.5	7.3 - 7.7
Total phosphorus (ug L^{-1})***	27.66	9.70 - 95.54	21.1	13.3 - 31.2	40.6	12.9 - 96.5
Landscape variables						
Distance to water source (m) **	322	40 - 858	404	40 - 816	713	46 - 1123

Table 3-2. Summary statistics of principle component analyses applied to 23 aquatic invertrebrates and 6 environmental variables in 36 nesting area ponds in the Whooping Crane nesting area and 5 temporal ponds sampled monthly (June - August).

	PC 1	PC 2
Nesting Area Ponds - Invertebrate ordination		
Eigenvalues	3.3	2.9
Percentage of Variance	15.2	13.2
Nesting Area Ponds - Environmental ordination		
Eigenvalues	1.8	1.4
Percentage of Variance	29.9	22.7
'Temporal Ponds' - Invertebrate ordination		
Eigenvalues	5.4	2.6
Percentage of Variance	31.9	15.1

Table 3-3. Environmental characteristics from June - August, 1999, for five temporal ponds and for nesting area pond 3A sampled in 1998 (3A-98) and 1999 (3A-99).

Variables	June		July		August		Pond 3	
	Mean	Range	Mean	Range	Mean	Range	1998	1999
Morphometric variables								
Area (ha)	0.44	0.03 - 0.64	0.64	0.03 - 0.95	0.64	0.03 - 0.95	0.64	0.64
Depth (cm)	34	23 - 46	26	13 - 36	14	2 - 21	25	37
Chemical Variables								
Conductivity (uS cm^{-1}) *	-	-	1541	1411 - 1700	-	-	1750	2570
pH	-	-	-	-	-	-	7.44	7.81
Total phosphorus (ug L^{-1})	19.97	17.75 - 21.37	20.44	15.92 - 26.51	30.86	15.06 - 43.17	49.78	15.40
Landscape variables								
Distance to water source (m)	-	-	-	-	-	-	100	100

*Conductivity only measured once for temporal ponds

Figure 3-1. Geographical location of Wood Buffalo National Park.

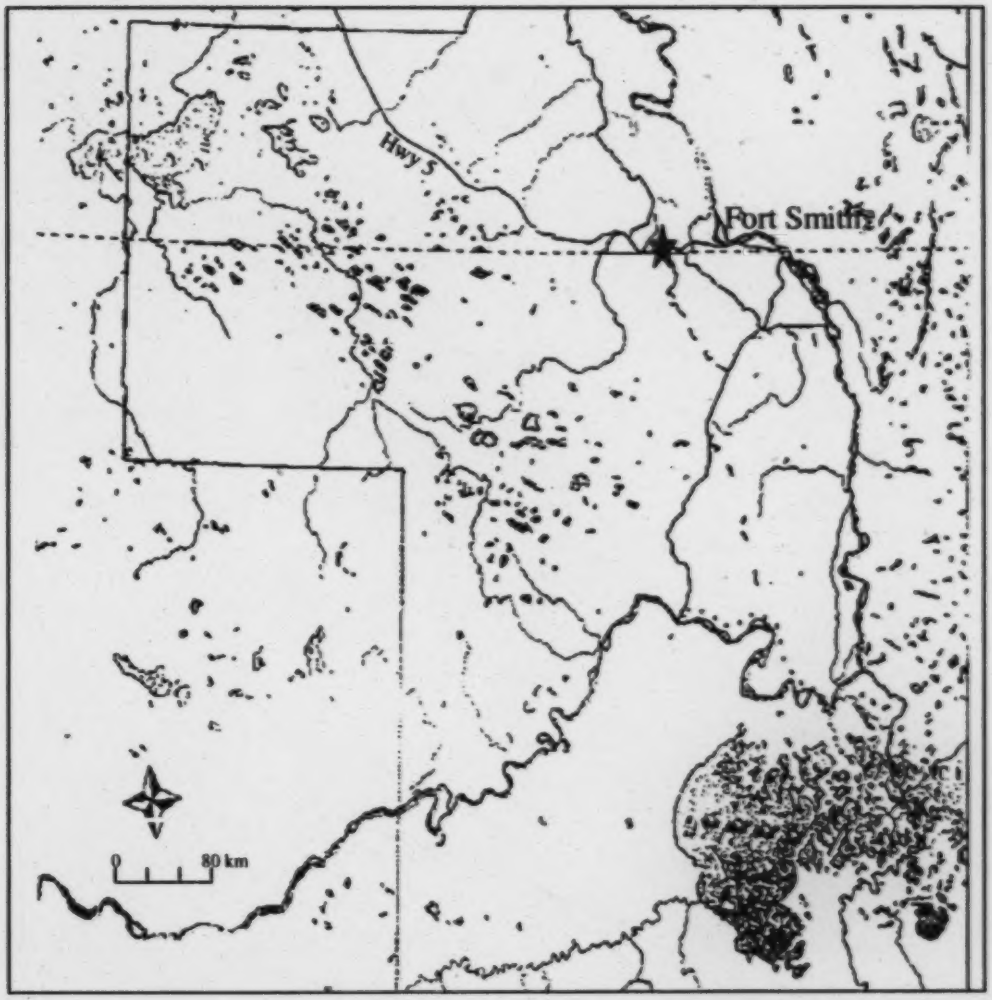


Figure 3-2. Map of Wood Buffalo National Park depicting location of temporal pond study (★).

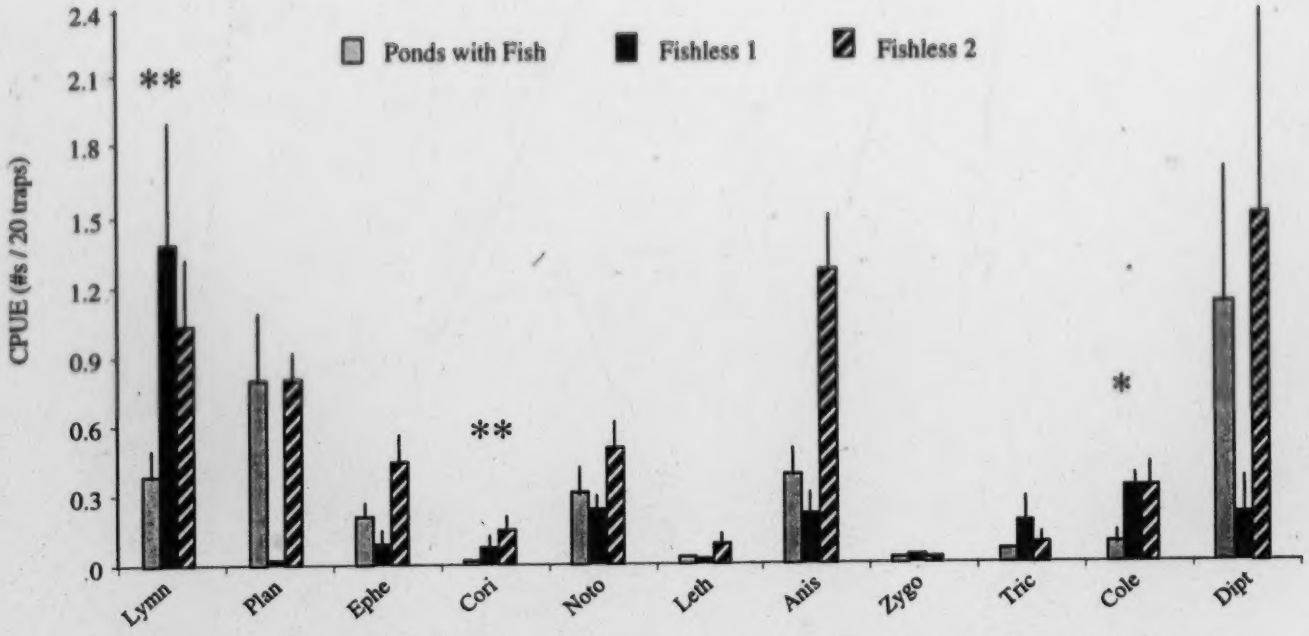
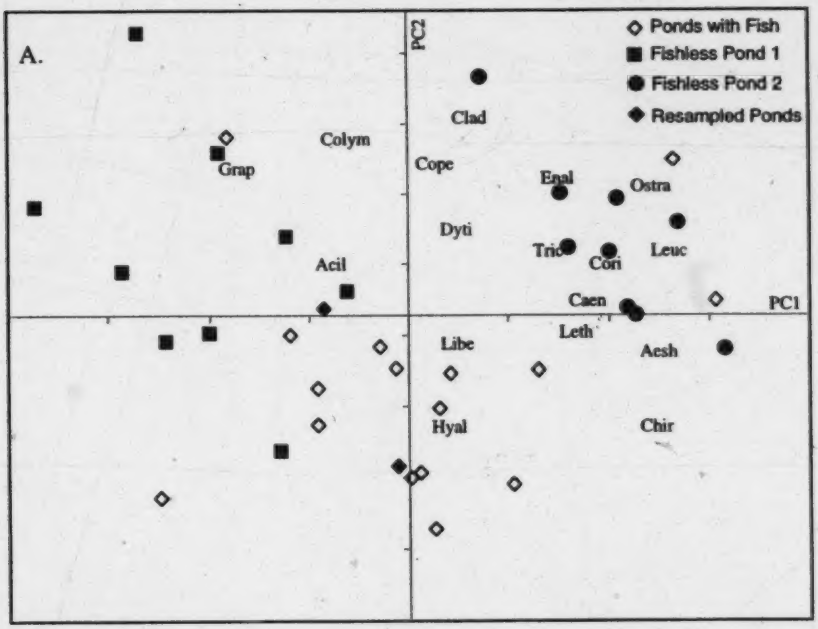
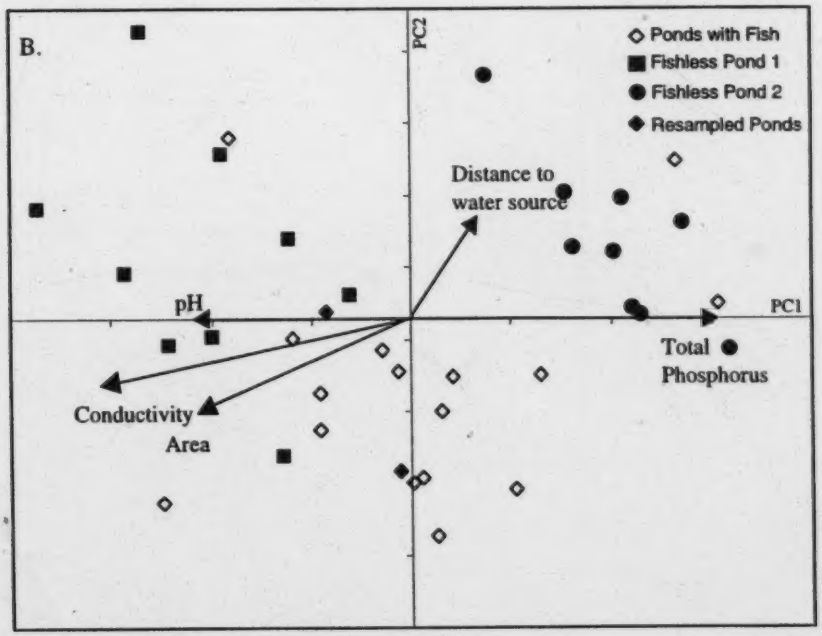


Figure 3-3. Relative abundance (±SE) of major macro-invertebrate taxa in 36 ponds in the Whooping Crane nesting area. Ponds are placed into 3 groups (Fish, Fishless 1, Fishless 2) based on th[illegible]omposition of their invertebrate communities. Asterisks denote significant differences between fis[illegible]d fishless groups by two-sample t-tests (*P<0.10, **P<0.05). Abbreviations found in Appendix B.

Figure 3-4. PCA of invertebrate communities from 36 ponds within the wetland complex of Wood Buffalo National Park (A) ordination overlays of taxa eigenvector, abbreviations are found in Appendix B. (B) ordination with plots of selected environmental variables. Vectors (arrows) point in direction of increasing values, with longer vectors indicating stronger correlations between the two PC axes and the vector variable.


A.
PC2
PC1
Ponds with Fish
Fishless Pond 1
Fishless Pond 2
Resampled Ponds
Clad
Colym
Grap
Cope
Enal
Ostra
Dyti
Trio
Cori
Leuc
Acil
Caen
Leth
Libe
Aesh
Hyal
Chir


B.
PC2
PC1
Ponds with Fish
Fishless Pond 1
Fishless Pond 2
Resampled Ponds
Distance to
water source
pH
Total
Phosphorus
Conductivity
Area

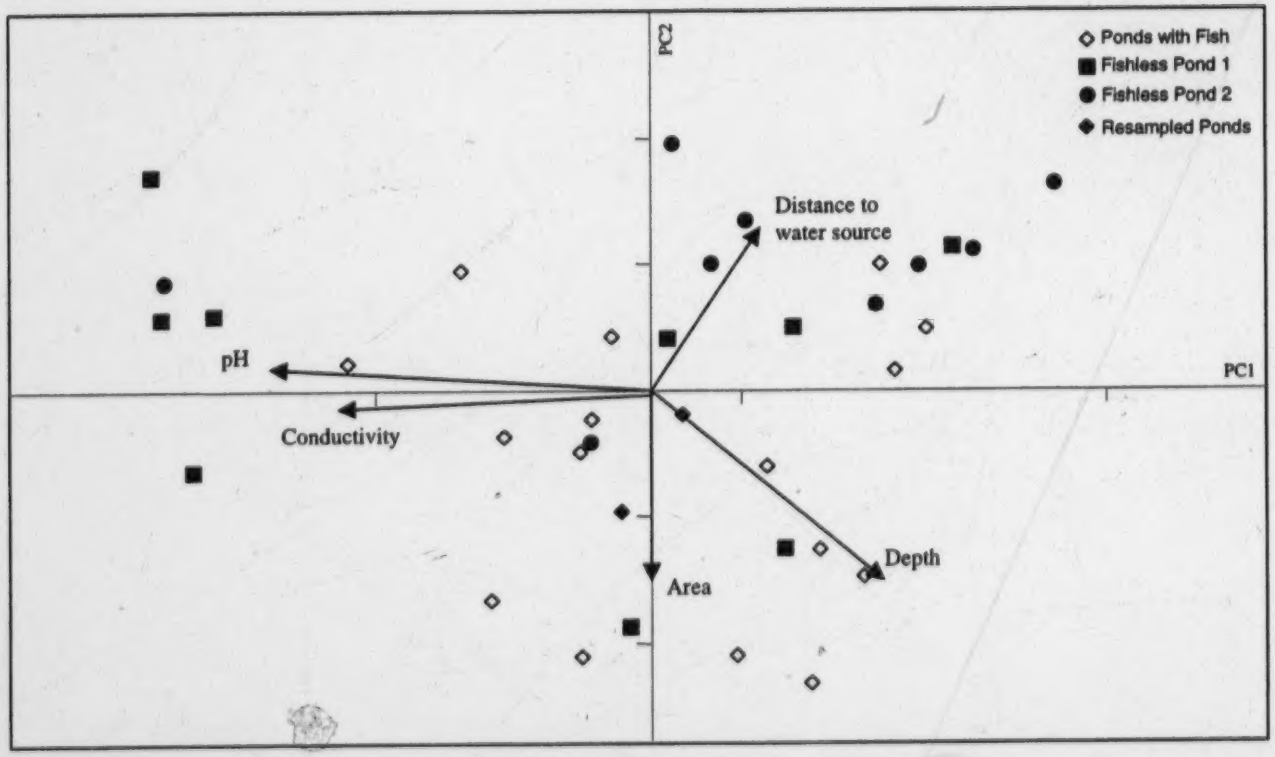


Figure 3-5. PCA of environmental variables for 36 ponds within the Whooping Crane nesting area. Ponds are placed into 3 groups (Fish, Fishless 1, Fishless 2) based on the composition of their invertebrate communities.

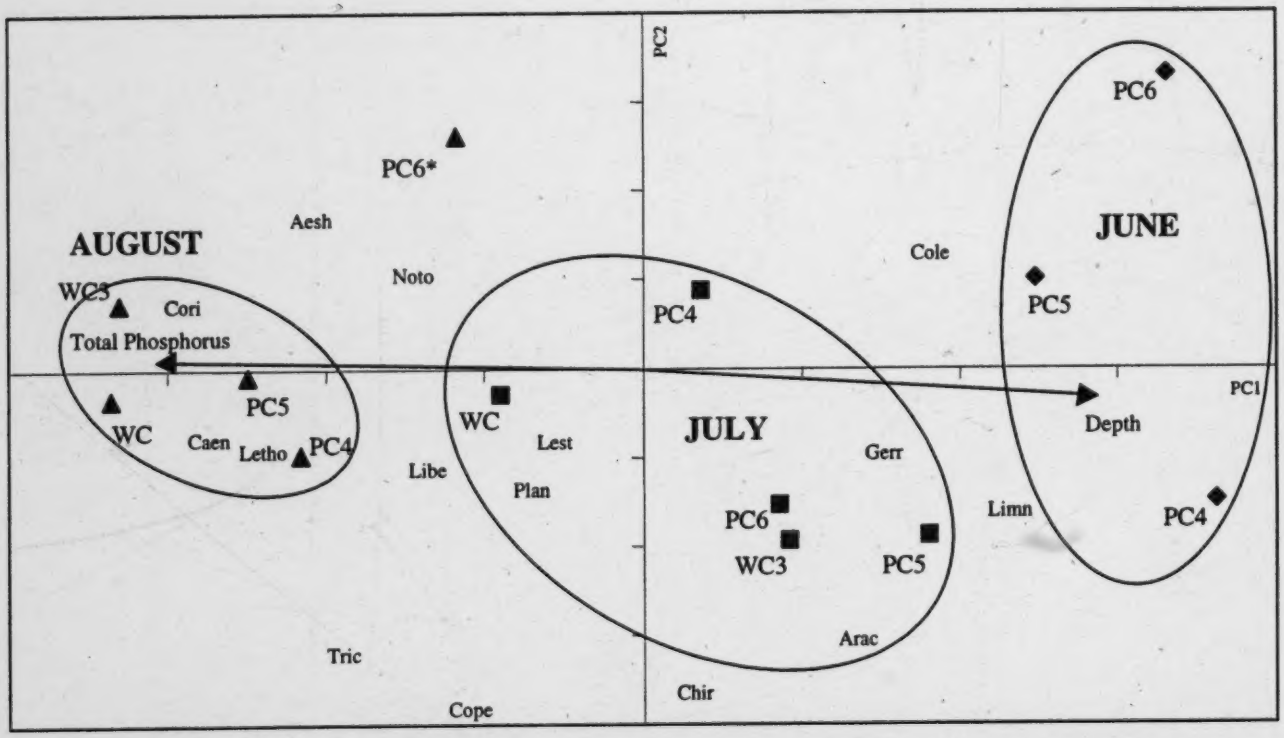


Figure 3-6. PCA joint plot of invertebrate communities and environmental variables from five temporal ponds sampled in June (◆), July (■), and August (▲), 1999, with taxon eigenvector values overlayed. Asterisk denotes site/time period that fish were present. Abbreviations for invertebrate taxa found in Appendix A.

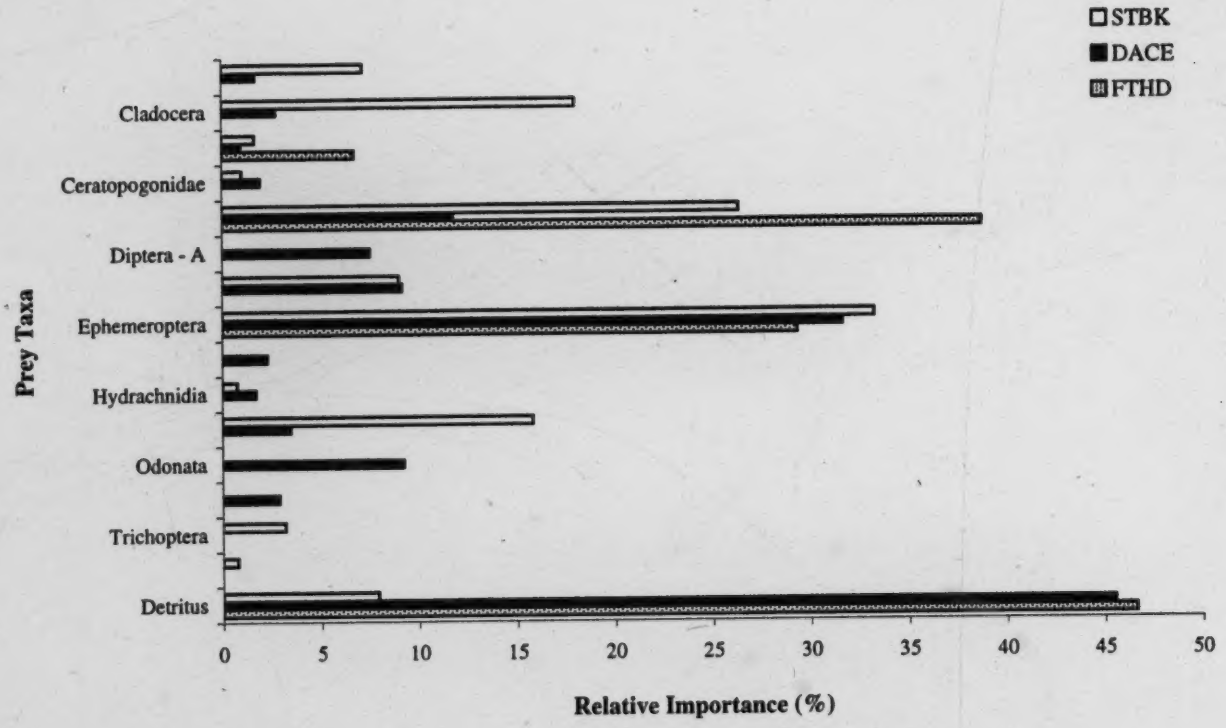


Figure 3-7. Relative Importance (%) of prey for STBK, DACE, and FTHD in the wetland complex of Wood Buffalo National Park. Diptera - A and Diptera- P indicate Diptera, other than Chironomidae and Ceratopogonidae, found in adult and pupal stages of development, respectively.

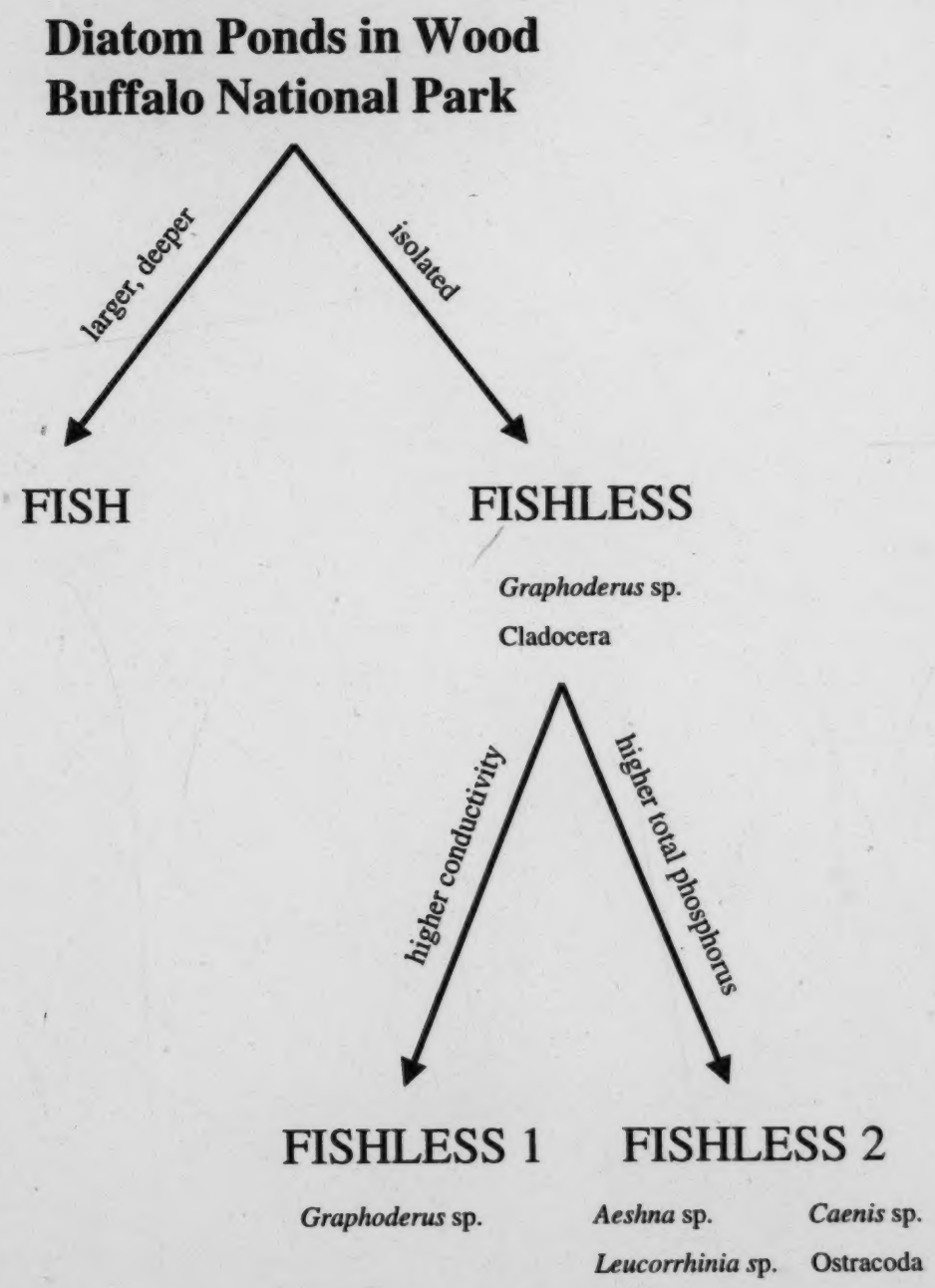


Figure 3-8. Summary flow chart of assemblage types and environmental data for distinct pond assemblages within WBNP. Species significance determined by indicator species test, significance of environmental factors determined from t-tests.

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Chapter IV. A STABLE ISOTOPE EVALUATION ON THE STRUCTURE OF POND FOOD WEBS IN THE WHOOPING CRANE NESTING AREA, WOOD BUFFALO NATIONAL PARK, CANADA

INTRODUCTION

Nearly inaccessible wetlands in Wood Buffalo National Park (WBNP), Alberta-Northwest Territories, provide the nesting habitat for the last migratory population of Whooping Crane, *Grus americana*. Since this nesting habitat was discovered in the 1950s, conservation efforts have increased the population of this once widely distributed bird from 16 individuals in 1941 to nearly 200 today (B. Johns, Canadian Wildlife Service, *pers. comm.*) The revised plan of the Canadian Whooping Crane Recovery Team calls for a stabilisation or increase in the existing WBNP population, and the establishment and support of two other migratory populations by 2020 (Edwards et al. 1994). To achieve these objectives, the recovery plan identifies as a major priority improved understanding of the food and habitat requirements of whoopers in WBNP, including information on the wetland food web that supports the adult cranes and their chicks.

In response, WBNP recently permitted a limited number of studies to be conducted in this restricted area, designated as Zone 1 – Special Preservation Area, protected under the National Parks Act. Focusing on vegetation and landscape structure, Timoney et al. (1997) noted that whoopers prefer deeper "diatom ponds", which are themselves a rare type of boreal wetland. These small (10 to >1000 m in diameter) ecosystems are distinct from other wetlands in the area, having greater amounts of open water and less terrestrial vegetation. They are commonly associated with reeds, sedges and bulrushes, and contain shallow, clear water virtually devoid of plankton; instead, benthic diatoms dominate the within-pond primary producer community. Most ponds are fed by groundwater and are hydrologically isolated from local streams, although they are subject to spring flooding. Water levels, which likely influence the resident biota, vary both seasonally and among ponds. Echoing the revised recovery plan, Timoney et al. (1997) recommended that research be focused on this wetland habitat and especially on relationships among habitat characteristics, structure of the food web, and the summer diet of the Whooping Crane.

Because of restricted access to the nesting area, past research on crane trophic ecology has concentrated on the wintering grounds (Aransas National Wildlife Refuge), identifying many coastal prey that are absent from WBNP (Hunt and Slack 1987, Chavez-Ramirez 1996). Until this recent initiative, there have been no direct studies of the crane's summer diet, with older studies listing only potential food items (Allen 1956, Novakowski 1966). Likewise, the food webs of the diatom ponds are virtually unknown.

Preliminary findings from an ongoing study suggested a major dichotomy within this wetland system (D. Bergeson, Parks Canada & University of Alberta, *pers. comm.*): nearly all ponds in which cranes were observed to forage contained small-bodied fishes, such as brook stickleback (*Culaea inconstans*) and several minnows (Cyprinidae): fathead minnows (*Pimephales promelas*), northern redbelly dace (*Phoxinus eos*), pearl dace (*Margariscus margarita*), and finescale dace (*Phoxinus neogaeus*). In contrast, most adjacent ponds in which cranes did not feed were fishless. This strongly suggested that fish are an important trophic link between whoopers and their habitat. If so, to what extent do fish contribute directly or indirectly to the trophic ecology of cranes? What is the food web supporting these small-bodied fish populations? How are 'fishless' food webs structured to make them unsuitable or less desirable for crane foraging? In the absence of studies on the diatom ponds, these questions could not be answered.

Conventional methods of studying these aquatic food webs and their links to Whooping Cranes can not be fully applied to the rare and sensitive diatom pond ecosystem, where intensive studies throughout the summer are not permitted and the costly access to these remote sites. Stable isotope analysis (SIA) has emerged as a valuable tool for investigating aquatic food webs (Fry 1991) and should provide a viable alternative for integrating and understanding the structure and function of the diatom pond food webs. In particular, stable isotope ratios of carbon (^{13}C:^{12}C) and nitrogen (^{15}N:^{14}N) can be used to obtain a time-integrated picture of energy sources and trophic levels, respectively, based on long-term assimilated diets. For use with the small-bodied fishes, however, stomach content analysis (SCA; see Chapter III) provides a degree of taxonomic precision that can not be obtained by SIA, thus making SCA and SIA highly complementary approaches to food web analysis (Gearing 1991, Vander Zanden et al. 1998, Harvey and Kitchell 2000, Beaudoin et al. 2001).

The isotopic composition of an organism is a time-integrated measure of its diet (DeNiro and Epstein 1978, 1981, Fry 1988, Wada et al. 1993, Gannes et al. 1997). Consumers typically have stable carbon and nitrogen isotopic values that are less negative and more positive, respectively, relative to their prey because lighter isotopes (i.e., ^{14}N and ^{12}C) are lost via preferential excretion or respiration and fixation (Macko et al. 1982, Minagawa and Wada 1984, Keough et al. 1996). Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios exhibit a consistent increase of approximately 0-1‰ and 3-5‰, respectively, with each addition in trophic level (DeNiro and Epstein 1978, 1981, Minagawa and Wada 1984, Peterson and Fry 1987). Differences in diet and subsequent changes in the ratios of heavy to light isotopes of certain elements (i.e., isotope fractionation) lead to distinct isotopic values among organisms that can allow researchers to trace the origin of organic carbon of organisms and identify their trophic positions within a food web (Peterson and Fry 1987, Gannes et al. 1998).

A major advantage in using SIA for this study is the ability to use tissues obtained through non-lethal sampling (Hobson et al. 1996). Feathers of Whooping Crane are lost naturally and frequently on the nesting grounds; although they are metabolically inactive, their isotopic composition reflects the diet of their owner at the time of feather formation (Hobson and Clark 1992). Preliminary analyses of whooping crane feathers (using isotopic ratios of deuterium) collected in WBNP suggested that feather formation occurs on the breeding grounds and not the wintering grounds (Duxbury and Holroyd 1996). Therefore, collection of feather samples is a benign but effective approach to establish the position of cranes in the pond food webs and to evaluate the trophic relationships among cranes, fish, and other organisms.

The general objective of this study was to investigate the food webs of diatom ponds in the Whooping Crane nesting area. In particular, my study focused on a comparison of food webs that contain and lack fish, the food sources on which fish rely, and the sources of primary production that drive these ecosystems. Specifically, using SIA, I (i) compared the structure of food webs of ponds containing and lacking fishes, (ii) established the trophic positions of fish and cranes in the food webs, (iii) determined temporal isotopic signature changes in pond ecosystems, and (iv) assessed the relative importance of internal versus external carbon in the flow of energy through the pond food

webs. To help interpret food web structure from SIA, limited results of SCA are also presented here; more detailed SCA are found in Chapter III.

METHODS AND MATERIALS

For description of sites and general methodology, refer to "*Description of Study Site*" and "*Field & Laboratory Methodology*" in the methodology section of Chapter III.

Selection of Study Sites

From June to August of 1998 and 1999, a total of 36 ponds were sampled, and a sub-sample was analysed for SIA. Seven ponds within the nesting area of the Whooping Crane were selected for SIA based on their fish assemblages (Table 4-1). Pairs of ponds, one containing fish and the other fishless, were chosen for SIA from the territories of 3 pairs of birds (Table 4-1). Of the four fishless ponds chosen, subsequent analyses revealed that three contained the Fishless 1 invertebrate community and one contained the Fishless 2 community (see Chapter III). Because of logistic limitations, ponds within the nesting area were only sampled once, with the exception of one pond (referred to as 3A-98 and 3A-99) sampled in both 1998 and 1999 (Table 4-1). Therefore, to evaluate seasonal variation in the wetland system, a pond outside the nesting grounds was sampled monthly (June – August) basis in 1999. This "temporal pond" was located where Highway 5 crosses Preble Creek and was selected due to accessibility and similarity to nesting-area ponds.

Field and Laboratory Methodology

a) Aquatic Biota, Carbon Sources, and Environmental Variables

Aquatic taxa representing all trophic levels were sampled from each pond. Whooping Crane feathers from six nesting areas were collected, washed in deionized distilled water, and frozen for subsequent SIA.

Fish were caught in minnow traps and activity traps (Murkin et al. 1983) that were set for 1-2 hours (for SCA) or overnight (for SIA). Upon capture, fish were identified as brook stickleback (STBK), fathead minnow (FTHD), or the composite group

DACE (see Chapter III), counted, and a subset of several size-classes was sacrificed and frozen for further processing.

Aquatic macro-invertebrates were collected from each pond using minnow traps, activity traps, and pond nets, and identified to broad taxonomic groups in the field. Benthic fauna was sampled at three sites within each pond by taking a 500-mL core of the surface sediment. Snails and other epiphytic invertebrates were collected by hand. A sub-sample of invertebrates was brought back to the laboratory alive, separated from sediment, vegetation and detritus, sorted, and held in water for at least 24 h to allow voiding of guts for subsequent SIA. Zooplankton samples were obtained from three horizontal hauls of a 243-µm tow net through the shallow water column. Pooled samples were examined under a dissecting microscope and sorted by hand into three groups, Cladocera, Copepoda, and Ostracoda. However, only pond 1B produced a sample (of Copepoda) of sufficient biomass to conduct SIA.

Phytoplankton samples were obtained from three horizontal tows using a 64-µm net. Even after pooling, however, phytoplankton samples from individual ponds did not produce sufficient biomass for SIA. The top 1cm of sediment, collected with a hand-held corer, was used for SIA of benthic algae. Particulate organic matter (POM; <64 µm) was collected by filtering 1 L water through the phytoplankton net onto a precombusted GF/C filter. Care was taken not to contaminate filters with other organic matter, mainly zooplankton, although rotifers were sometimes difficult to separate out. Filters were frozen for subsequent SIA. In 1999, water samples for measuring dissolved inorganic carbon (DIC) were collected in poly-propylene bottles, making sure no air bubbles were inside, and refrigerated for SIA. Periphyton was collected from submerged wood and frozen for later identification, although little dead fall was found within the ponds and these samples never produced enough biomass for SIA. Emergent, submergent and floating macrophytes were collected by hand. Terrestrial samples (dominant plants and soil) that represent potential external carbon sources were collected from the surrounding shoreline of each pond. For both terrestrial and aquatic plant samples, only the leaves were used for SIA to decrease within-organism variability (Gearing 1991). Plant samples were washed vigorously to remove algae or other contaminants before being frozen for later SIA.

b) Stable Isotope Analysis

Following collection, all samples were sorted and frozen for shipment to University of Alberta for detailed identification. With fish, a portion of the dorsal white muscle tissue was removed. If insufficient muscle tissue was available, fish were eviscerated, decapitated, and skinned, prior to analysis (see Chapter II). Samples containing a carbonate fraction (invertebrates, plant material, soil, and sediment), which can confound isotopic signatures, were soaked in 1N HCl for approximately 24 h (or until bubbles no longer appeared) to remove inorganic carbon, and then rinsed in deionized distilled water. Lipids were not extracted from biotic samples since lipid extraction strongly affects the isotopic signatures of fish (Chapter II) and invertebrates (Neary et al., unpublished). Following these treatments samples were air dried and ground to a fine powder using a mortar and pestle.

All SIA were conducted at the National Water Research Institute, Saskatoon, Canada. Dried samples were analysed for stable isotope ratios of carbon and nitrogen using a Micromass Optima™ EA online continuous-flow isotope ratio-mass spectrometry (CF-IRMS) coupled with an Carlo Erba NA1500 elemental analyser (EA) and Autosampler. For fish and invertebrates, approximately 1 mg of sample was used, whereas 8 mg was used for sediment, soil, and aquatic and terrestrial plants. Dried, homogenous samples were loaded into 5 x 8 mm tin capsules and introduced into a combustion furnace where they were converted to a gaseous product (CO_2 and N_2) at 1030 °C. A helium carrier gas stream transported the sample gases through a Cu column at 450 °C to reduce oxygen and nitrogen oxides, a water trap to remove water, and through a low efficiency gas chromatograph column that separated CO_2 and N_2. A pulse of CO_2 and N_2 reference gas was introduced into the mass spectrometer with an automated gas injection system, followed by the CO_2 and N_2 sample gas. An internal working protein standard ($\delta^{13}C = -12.6 \pm 0.1$, $\delta^{15}N = 5.6 \pm 0.1$) was used to determine sample repeatability, and corrected relative to international standards (PDB and AIR).

Water samples for DIC analysis were transferred to glass serum bottles for isotopic analysis. Samples were converted to CO_2 by acidification *in vacuo* with 100% phosphoric acid for the analysis of stable carbon isotope ratios. After the dissolved CO_2

had exsolved within the headspace gas of the bottle, a portion of this gas was sub-sampled and injected into a gas chromatograph/combustion furnace/isotope-ratio mass spectrometer (GC/IRMS) system to determine the carbon isotope ratio of the CO_2, following CF-IRMS analysis procedures described above. The isotopic values of the samples were determined in the GC/IRMS by comparison with a reference CO_2 gas of known isotope ratio ($\delta^{13}C = -12.5‰$), which was introduced from a reservoir to the IRMS.

POM samples were analysed for stable carbon ratios on a Micromass Optima™ dual-inlet isotope ratio mass spectrometer (Boutton et al. 1983). Samples for carbon analysis were sealed into an evacuated length of a 9-mm Vycor combustion tube along with 2 g CuO (acting as an oxidant) and baked at 850 °C for 3 h. Following combustion, samples were slowly cooled to room temperature and CO_2 was measured from the combusted samples using a mass spectrometer. A working laboratory standard of graphite (USGS 24: $\delta^{13}C = -16.05‰$) was run after every 10 samples to determine sample repeatability.

Isotope ratios are expressed in delta (δ) notation as parts per thousand (per mil, ‰) differences from a standard as follows:

$$\delta^{13}C \text{ or } \delta^{15}N\ (‰) = [(R_{sample} - R_{standard})/R_{standard}] \times 1000$$

where R denotes $^{13}C/^{12}C$ or $^{15}N/^{14}N$. All results are reported relative to internationally calibrated reference material, Pee Dee Belemnite (PDB) limestone standard for $\delta^{13}C$ and atmospheric nitrogen (AIR) for $\delta^{15}N$.

c) Stomach Content Analysis

For the analysis of fish diets, a sub-sample of fish collected within the ponds was used. The complete digestive tract was removed from each fish, contents were sorted and identified to the lowest possible taxonomic level. The frequency of occurrence for each prey taxon was calculated for each fish taxon as a proportion of stomachs containing the particular prey taxon (Bowen 1996).

RESULTS

Primary Producers

Terrestrial inputs were consistent between paired ponds for $\delta^{13}C$ (-33.8 to –25.84‰) and $\delta^{15}N$ (-4.4 to 4.6‰; Figures 4-1 – 4-3). Signatures were typically too positive or too negative to suggest that these inputs acted as important food sources for invertebrates within the systems, though some samples of Lymnaeidae, Ephemeroptera, Hemiptera, Coleoptera, and Diptera may have used this source (Figures 4-1 – 4-3).

Isotopic signatures of emergent macrophytes, such as Bulrush (*Scirpus* sp.) and sedge (*Carex* sp.), were too positive within ponds containing fish to suggest them as an important food source for invertebrates (Figures 4-1A, 4-2A, 4-2C, 4-3A). Within some fishless ponds, isotopic signatures of emergent macrophytes placed them as potential energy sources for some invertebrates, including Corixidae, Lymnaeidae, and Chaoboridae (Figures 4-2B, 4-2D, 4-3B). Bladderwort (*Utricularia* sp.), a floating macrophyte typically found in ponds containing fish, were more positive in $\delta^{15}N$ (2.4 to 4.0‰; Figures 4-1A, 4-2A, 4-2C, 4-3A), as expected from their carnivorous habit. Their more negative $\delta^{13}C$ signatures, relative to other macrophytes, suggests that fish and invertebrates within these ponds may have used bladderwort as a food source, or it may reflect shared, common energy sources between the carnivorous plants and animals. Pond 1B was the only fishless pond that had bladderwort, though its isotopic signature ($\delta^{13}C$ = -31.4‰, $\delta^{15}N$ = 2.3‰) suggested few invertebrates used it as a food source (Figure 4-1B).

Pond sediment, composed of benthic diatoms, had isotopic signatures that were similar within groups of ponds (i.e., fish and fishless), although signatures in fishless ponds were consistently enriched in both $\delta^{13}C$ and $\delta^{15}N$ (Figures 4-1 – 4-3). Isotopic signatures of sediment suggest it may be a food source for a variety of animals, including Gastropoda, Anisoptera, Hemiptera, Diptera and tadpoles.

POM samples, which presumably contain a high proportion of bulk phytoplankton, were obtained from only half the ponds; nevertheless, signatures varied considerably between fish and fishless ponds, with the latter being more positive(Figures 4-2 – 4-3). Invertebrates that may have used POM as a potential food source include

Gastropoda (Figure 4-2C), although others may have used POM as well, if eaten in combination with another, more negative, carbon source.

Pond water samples exhibited a DIC isotopic signature ranging from –8.0 to –6.1‰ for ponds containing fish and –9.5 to –8.7 for fishless ponds (Appendix G).

Invertebrates

Overall, the invertebrate community of fishless ponds was consistently more positive in both $\delta^{13}C$ and $\delta^{15}N$ values than were invertebrates within ponds containing fish (Figures 4-1 – 4-3). Assuming a 3-4‰ enrichment of $\delta^{15}N$ between prey and predator, few invertebrate taxa had isotopic signatures that clearly indicated they were solely carnivorous. Invertebrates such as *Dolomedes triton*, Hirudinea, and Belestomatidae consistently had higher $\delta^{15}N$ values, relative to other invertebrates, indicating predominantly carnivorous diets (Figure 4-1A, 4-2C). Likewise, few invertebrates had consistently low $\delta^{15}N$ signatures that would suggest a pure herbivorous diet. Taxa that appear to be predominantly herbivorous, however, included Ephemeroptera, Amphipoda, and Limniphilidae (Figures 4-1 – 4-3).

Vertebrates

The isotopic signatures of fish indicated that they were at or near the top trophic level in the pond food webs, with diets consisting of aquatic invertebrates and primary producers (Figure 4-1A, 4-2A, 4-2C, 4-3A). DACE and STBK had similar isotopic signatures, suggesting potential prey that included Gastropoda, Anisoptera, Hemiptera, Trichoptera, Coleoptera, and Diptera (Figure 4-1A, 4-2A, 4-2C, 4-3A). FTHD had similar $\delta^{15}N$ signatures to DACE and STBK, although their $\delta^{13}C$ was more negative, suggesting an alternate, unknown carbon source (Figure 4-3A). According to SCA, Ephemeroptera, Chironomidae, Coleoptera, and Crustacea were frequent prey for these fish (Table 4-2), consistent with stable isotope findings.

A wood frog (*Rana sylvatica*) adult and tadpoles were collected from fishless Pond 3C (Figure 4-2D). Tadpoles had isotopic values that were more positive compared to the adult frog, although both appeared to have mixed diets of invertebrates and primary producers.

Fourteen Whooping Crane flight feathers were run for analysis of stable isotopes to determine their position within the aquatic food web. Cranes were at the top trophic level within the ponds, with $\delta^{15}N$ signatures similar to or exceeding fish, suggesting Coleoptera, Lymnaeidae, Anisoptera, Diptera, and tadpoles as potential food sources (Figures 4-1 – 4-3). Feathers showed considerably positive and variable isotopic signatures of $\delta^{13}C$ (-27.7 to -15.0‰) with respect to any primary producer sampled within the aquatic and surrounding terrestrial system, leaving the carbon source unresolved.

Temporal Ponds

Yearly Variations

Sediment, and terrestrial and aquatic vegetation in a nesting area pond sampled in both 1998 (Pond 3A-98; Figure 4-2A) and 1999 (Pond 3A-99; Figure 4-2C), had consistent isotopic signatures. The greatest change in primary producers involved POM, with $\delta^{13}C$ values 10‰ more positive in 1999 relative to the previous year (Figure 4-2A, 4-2C). POM in 1998 was too negative to be considered a potential food source for organisms sampled within that year, though in 1999, it may have been a potential source of food for Gastropoda (Figure 4-2A, 4-2C).

The isotopic signatures of invertebrates and fish within this pond remained consistent from 1998 to 1999 (Figure 4-2A, 4-2C). The pond contained STBK and DACE in both years, with potential prey including Notonectidae, Anisoptera, Colymbetinae, Trichoptera, Gastropoda, Gerridae, and Chironomidae (Figure 4-2A, 4-2C). The floating, carnivorous macrophyte, *Utricularia* sp., may also have been a potential food source for DACE.

Seasonal Variation

The pond that was sampled monthly in 1999 showed little change from June to August (Figure 4-4; only June and August are shown). POM within the pond showed slight increases in $\delta^{13}C$ from June to August (Figure 4-4), with Gastropoda possibly using POM as a food source in June (Figure 4-4A).

Invertebrates maintained a similar position within the food web as summer progressed, with isotopic signatures suggesting an omnivorous diet for the majority of taxa (Figure 4-4). The positive shift in $\delta^{13}C$ values of Gerridae in August suggest the use of an alternate carbon source in late summer (Figure 4-4B).

DISCUSSION

Primary Producers and Carbon Source

The energy supply for aquatic organisms originates from a diversity of sources, allochthonous, autochthonous, or a combination of both (Peterson 1999). Identifying specifically the primary source of organic matter fuelling an aquatic food web is difficult given the variety of potential source materials, isotopic composition, and the integration of source materials by organisms. Terrestrial plants have distinct carbon isotopic signatures related to their photosynthetic pathway (e.g., C_3, C_4, or CAM), making them easily distinguishable. Terrestrial plants surrounding the nesting area ponds in Wood Buffalo National Park had isotopic signatures of carbon fixed around that expected for C_3 plants, -31 to –26‰ (Hecky and Hesslein 1995), and showed little change between fish and fishless ponds.

The $\delta^{13}C$ values of benthic algae typically overlapped with the isotopic signatures of animals in both fish and fishless ponds, supporting other findings that show carbon isotopic signatures of aquatic animals to be consistent with an algal-based food web (Peterson and Howarth 1987, Keough et al. 1996). Some consumers, however, had $\delta^{13}C$ values that were inconsistent with sole dependence on benthic algae, suggesting the variable use of different primary producers, including some not readily identified. POM, a proxy for bulk phytoplankton, showed general isotopic signature patterns that paralleled differences between fish and fishless food webs, suggesting phytoplankton to be a relatively important source to the food webs.

The marked positive values of $\delta^{13}C$ in primary producers within fishless ponds suggests a change in the isotopic composition of source carbon available to the food webs or a variation in the proportional utilisation of HCO_3^- and CO_2 by producers as a result of changes in their relative abundance (Stephenson et al. 1984). The $\delta^{13}C$ value of DIC (dissolved CO_2 and HCO_3^-) within both fish and fishless ponds indicated values close to

atmospheric equilibrium ($\delta^{13}C$ = -7 to –8‰, Keeling et al. 1979) and not of the isotopically light biogenic (respiratory) carbon, further suggesting that these ponds are dominated by autochthonous inputs of organic matter.

Consumers

Within the ponds of WBNP, 3 – 4‰ enrichments in ^{15}N between distinct trophic levels were not often observed, suggesting omnivory to be prevalent in these nesting area ponds (Kling et al. 1992). This type of generalist diet may also contribute to variations in the $\delta^{13}C$ signatures of organisms among and within ponds (Kling et al. 1992, Beaudoin et al. 2001), given that the signatures of different food sources are integrated by consumers (Kiriluk et al. 1995).

Stable isotope results depicted fish (when present) and Whooping Cranes to co-occupy the top predatory level within the pond food webs, with invertebrates typically having lower $\delta^{15}N$ values. SIA of some crane feathers indicated potential food sources that include tadpoles, snails, dragonflies, and beetles, although the enriched $\delta^{13}C$ of many feathers from some ponds suggested additional, unidentified prey or prey from sites off the breeding grounds. In contrast, Duxbury and Holroyd (1996) suggested, based on SIA of crane feathers and aquatic organisms, that fish were the dominant prey in the diets of whoopers. At least part of the discrepancy may have resulted from their having to consolidate feathers, fish, and invertebrates from various ponds from several different crane territories, rather than match cranes and potential prey by the territory in which the feathers were found, as was done here.

Some variation in isotopic signals of feathers from the aquatic food web is expected since whoopers moult feathers one year after they are formed, therefore, the isotopic value of a feather represents the diet in the previous year and not the year they are moulted (i.e., collected). This, though, should explain only small differences in $\delta^{13}C$ since the temporal food web study suggested little or no change in the isotopic values of primary producers and aquatic organisms between years.

The large positive shift observed in the $\delta^{13}C$ of many crane feathers strongly suggests that marine elements from the wintering grounds were used in the formation of Whooping Crane feathers and not local elements from WBNP, as suggested by Duxbury

and Holroyd (1996). The carbon isotopic composition of marine plants (-24 to –3‰) is distinctly different from that of freshwater plants (-45 to –23‰), reflecting differences in the photosynthetic pathways of the plants (C3, C4, or CAM) and/or the source carbon of the system (Osmond et al. 1981, Fry and Sherr 1984, Peterson and Fry 1987). With the ratio of ^{13}C to ^{12}C exhibiting little or no fractionation during each trophic transfer, the $\delta^{13}C$ value of a consumer should be similar to that of its food source. With many of the Whooping Crane feathers exhibiting more positive $\delta^{13}C$ values than those of the freshwater organisms sampled from the nesting area ponds, it is likely that those feathers are expressing a marine signal and not one of freshwater origin.

The few feather samples that did have more negative $\delta^{13}C$ values, similar to those of the freshwater organisms sampled within the nesting area ponds, suggest a signal from the nesting area ponds. Although this difference in $\delta^{13}C$ among feathers (i.e., marine vs. freshwater) may relate to the age of the bird from which the feathers came, and thus when and where moulting occurred, much remains unclear. Therefore the summer diet of cranes can not be confidently assessed by the isotopic analysis of feathers alone until a better understanding of feather formation and isotopic composition is known.

With the exception of Territory 3, fish displayed isotopic signatures that typically placed them at a trophic level similar to Whooping Crane, with $\delta^{15}N$ values suggesting diets of invertebrates and primary producers. Brook stickleback and dace had $\delta^{13}C$ signatures that spanned similar ranges, suggesting similar diets, which was supported by SCA. The carbon-isotope signatures of fathead minnows were somewhat more negative, suggesting alternate food sources.

Invertebrates within the nesting area ponds showed distinct isotopic patterns with respect to the presence or absence of fish. The lower $\delta^{15}N$ values among invertebrates in ponds containing fish, suggesting that they occupy a lower trophic position in such ponds, could be a direct or indirect consequence of predatory activities of fish. Nevertheless, trophic relationships among invertebrates remained generally similar between fish and fishless ponds. Herbivores, such as Ephemeroptera and Trichoptera, consistently had lower $\delta^{15}N$ signatures within the food webs while omnivores and carnivores were found at higher trophic levels.

Differences in $\delta^{13}C$ signatures between invertebrates within fish and fishless ponds were also observed and reflected differences in primary producers. Specifically, the consistent positive $\delta^{13}C$ values of invertebrates within fishless ponds corresponded to the enrichment in ^{13}C for POM and benthic algae, as discussed above.

Temporal Variation in Isotopic Signatures

Temporal variation in stable isotope ratios is common, particularly for smaller organisms with more rapid tissue turnover rates (Harvey and Kitchell 2000). Based on data from the 1999 temporal pond sampled in June and August, however, as well as the nesting area pond sampled in both 1998 and 1999, isotopic signatures for aquatic animals and primary producers exhibited consistency both seasonally and annually. This suggests that SIA of samples collected from visits during an extensive survey can provide representative pictures of the aquatic food webs regardless of the time or order in which they were sampled.

Conclusions

The isotopic investigation of the nesting area ponds within WBNP has provided information previously unavailable using conventional techniques. Stable isotope analysis described sources of primary productivity and trophic positions of consumers and revealed some distinct differences in food webs containing and lacking fish. Differentiation between stable nitrogen isotopic signatures was likely the result of predatory pressures placed on lower trophic levels by fish, while observed shifts in $\delta^{13}C$ likely corresponded to changes in the carbon sources fuelling the food webs.

Stable isotope analysis also provided useful insight into the trophic positioning of Whooping Cranes, refuting earlier conjectures that whoopers were primarily granivorous, similar to their North American cousins, the Sandhill Cranes (*Grus canadensis*). Details of the summer diet still remain undescribed, however, as the isotopic values of feathers appear to incorporate some wintering ground component, thus, by themselves, can not fully depict the food sources used while on the breeding grounds.

Identification of community patterns and feeding interactions within the nesting area ponds of Wood Buffalo National Park has provided valuable information on a

unique habitat that is virtually unknown. Moreover, results should assist the Recovery Team in assessing suitable habitat for range expansion in and around WBNP, as well as for the reintroduction of this magnificent bird elsewhere in North America.

Table 4-1. List and codes of study ponds and composition of their fish assemblages. Dace is a composite taxon consisting of northern redbelly, finescale, and pearl dace, plus hybrids.

Territory	Pond	WBNP ID #	Fish Assemblages	Thesis ID
1	A	4.1.98	Brook stickleback	1A
	B	4.2.98	Fishless 2*	1B
3	A	8.1.98	Brook stickleback Dace	3A-98
	B	8.2.98	Fishless 1*	3B
	A	1.2.99	Brook stickleback Dace	3A-99
	C	1.1.99	Fishless 1*	3C
13	A	11.2.99	Brook stickleback Dace Fathead minnow	13A
	B	11.1.99	Fishless 1*	13B

* Fishless 1 and Fishless 2 identify the two types of fishless ponds, based on invertebrate community composition, refered to in Chapter III.

Table 4-2. Percent frequency of occurrence (%FO) of prey taxa consumed by fish taxa in nesting area ponds of Wood Buffalo National Park. Dace is a composite group that includes northern redbelly, finescale, and pearl dace, plus hybrids.

Fish	Prey Taxa	%FO
Brook Stickleback (n=30)	Copepoda	13.3
	Cladocera	53.3
	Coleoptera	6.7
	Ceratopogonidae	3.3
	Chironomidae	83.3
	Diptera - P*	30.0
	Ephemeroptera	76.7
	Hydrachnidia	3.3
	ostracoda	60.0
	Trichoptera	13.3
	Eggs	3.3
	Detritus	6.7
Dace (n=30)	Copepoda	6.7
	Cladocera	6.7
	Coleoptera	3.3
	Ceratopogonidae	6.7
	Chironomidae	33.3
	Diptera - A*	16.7
	Diptera - P*	26.7
	Ephemeroptera	53.3
	Gastropoda	3.3
	Hydrachnidia	6.7
	ostracoda	10.0
	Odonata	23.3
	Hemiptera	10.0
	Detritus	36.7
Fathead Minnow (n=4)	Coleoptera	25.0
	Chironomidae	75.0
	Ephemeroptera	50.0
	Detritus	25.0

* Diptera - A and Diptera - P indicate Diptera, other than Chironomidae and Ceratopogonidae, found in adult and pupal stage of development, respectively.

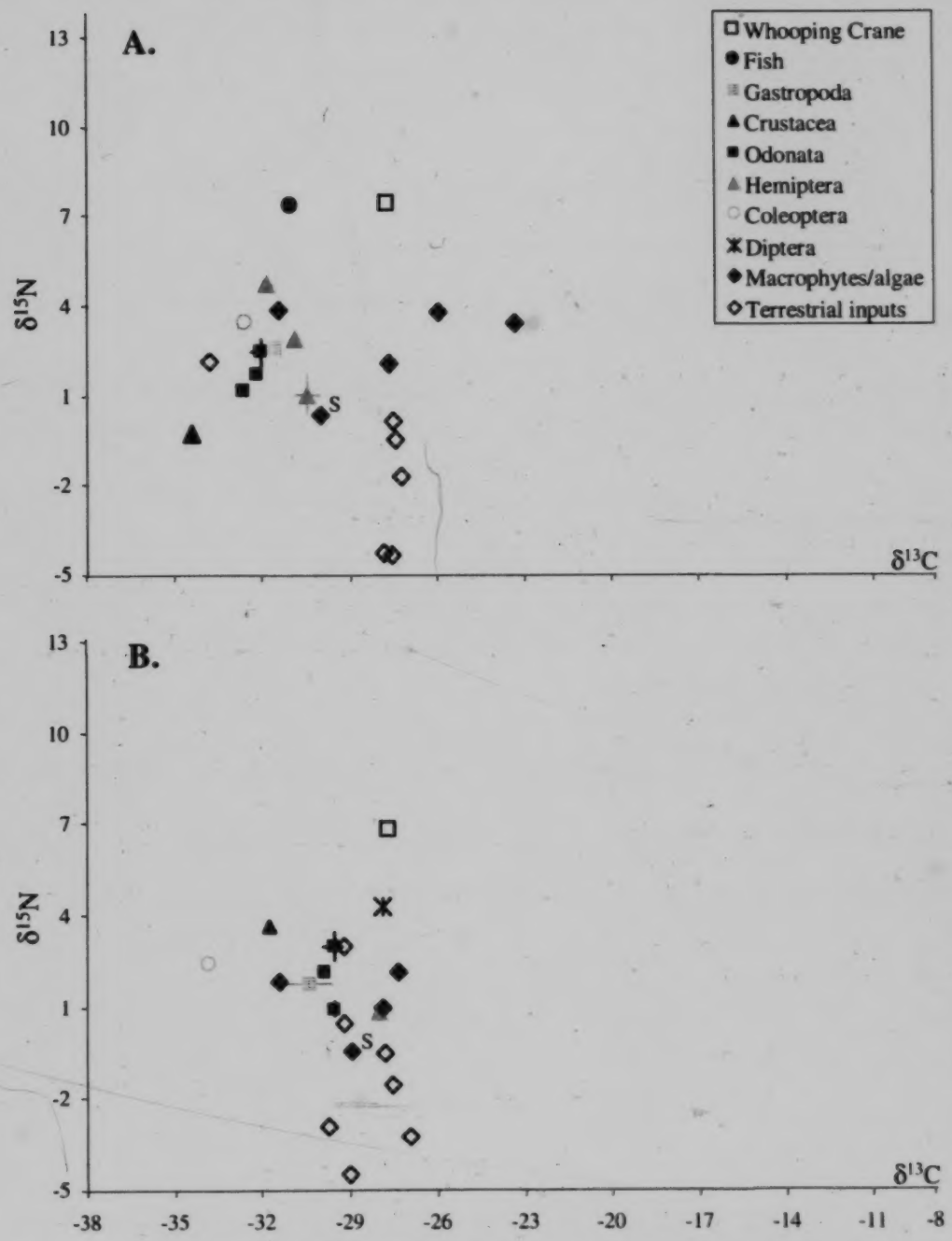

Figure 4-1. Scatter plot of $\delta^{15}N$ and $\delta^{13}C$ signatures (‰) of common taxa collected in 1998 from (A) ponds 1A, with fish, and (B) pond 1B, Fishless 2. When possible, mean (±SE) values are plotted. Crane data is from territory, could not be assigned to specific pond; value repeated in A & B. Values and organisms listed in Appendix D, E, and F. S denotes pond sediment/benthic algae.

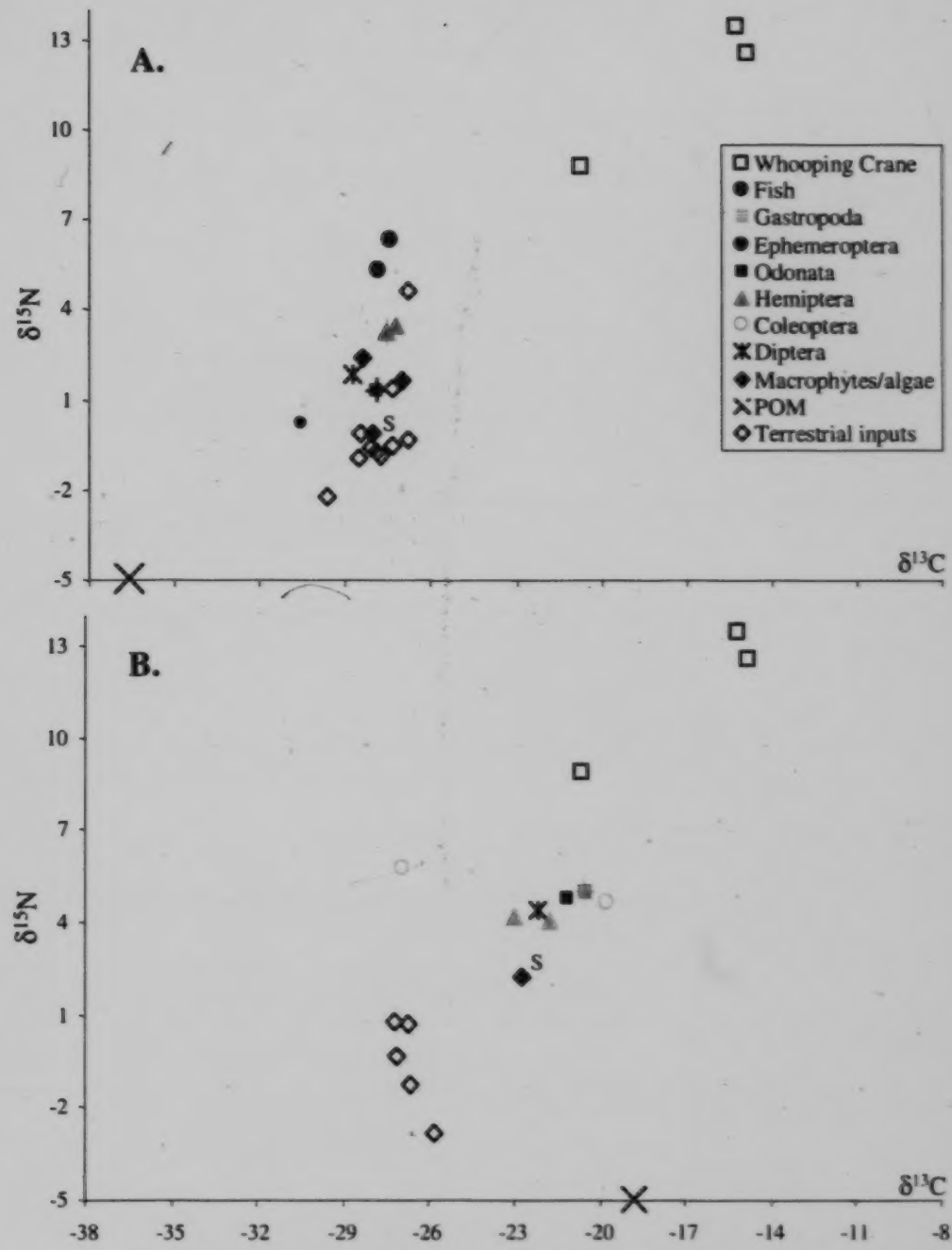

Figure 4-2. Scatter plot of $\delta^{15}N$ and $\delta^{13}C$ signatures (‰) of common taxa collected in 1998 from (A) pond 3A-98, with fish, and (B) pond 3B, Fishless 1. When possible, mean (±SE) values are plotted. Crane data is from territory, could not be assigned to specific pond; value repeated in A & B. Values and organisms listed in Appendix D, E, and F. S denotes pond sediment/benthic algae.

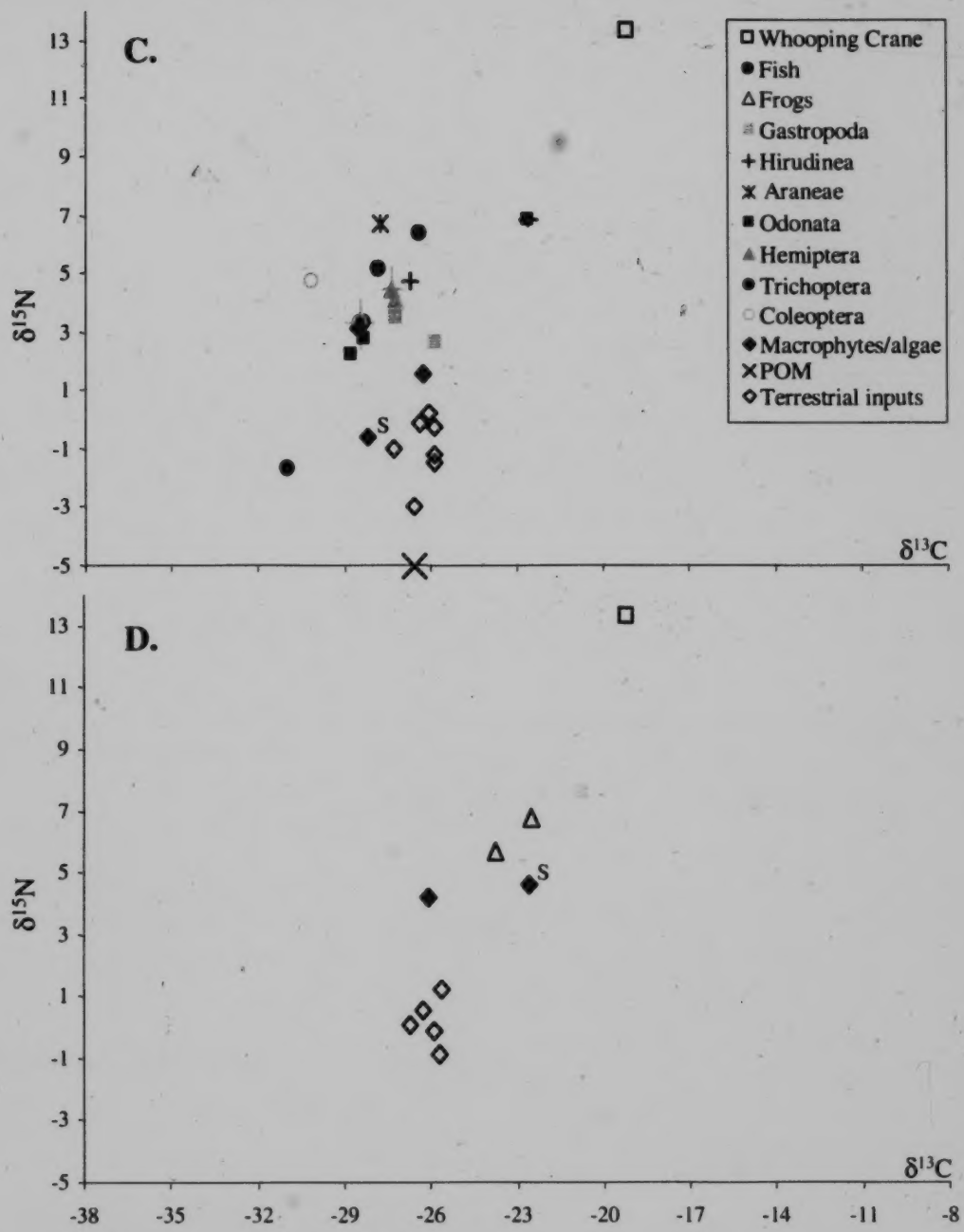

Figure 4-2 (continued).

Scatter plot of $\delta^{15}N$ and $\delta^{13}C$ signatures (‰) of common taxa collected in 1999 from (C) pond 3A-99, with fish, and (D) pond 3C, Fishless 1. When possible, mean (±SE) values are plotted. Crane data is from territory, could not be assigned to specific pond; value repeated in A & B. Values and organisms listed in Appendix D, E, and F. S denotes pond sediment/benthic algae.

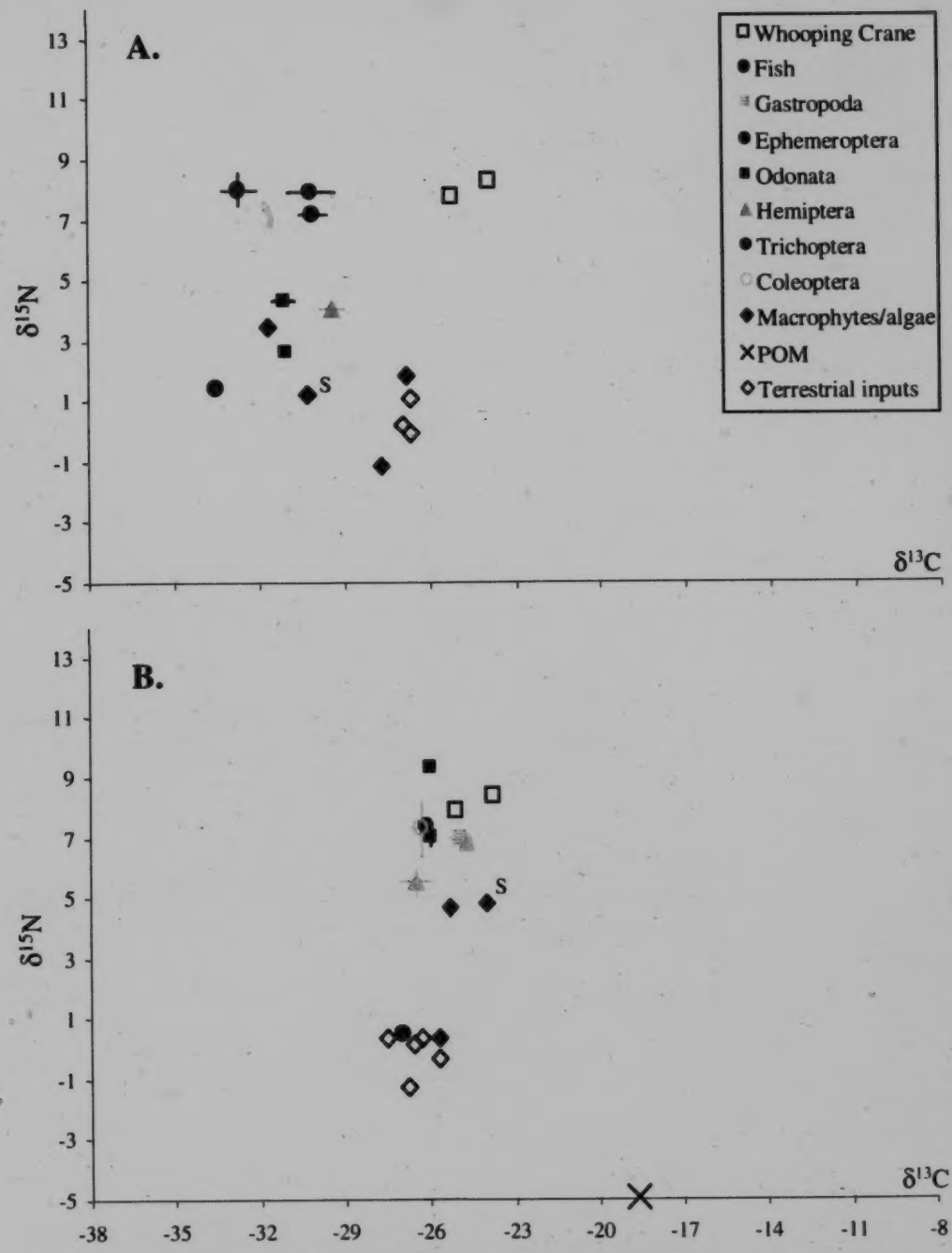

Figure 4-3. Scatter plot of $\delta^{15}N$ and $\delta^{13}C$ signatures (‰) of common taxa collected in 1999 from (A) pond 13A, with fish, and (B) pond 13B, Fishless 1. When possible, mean (±SE) values are plotted. Crane data is from territory, could not be assigned to specific pond; value repeated in A & B. Values and organisms listed in Appendix D, E, and F. S denotes pond sediment/benthic algae.

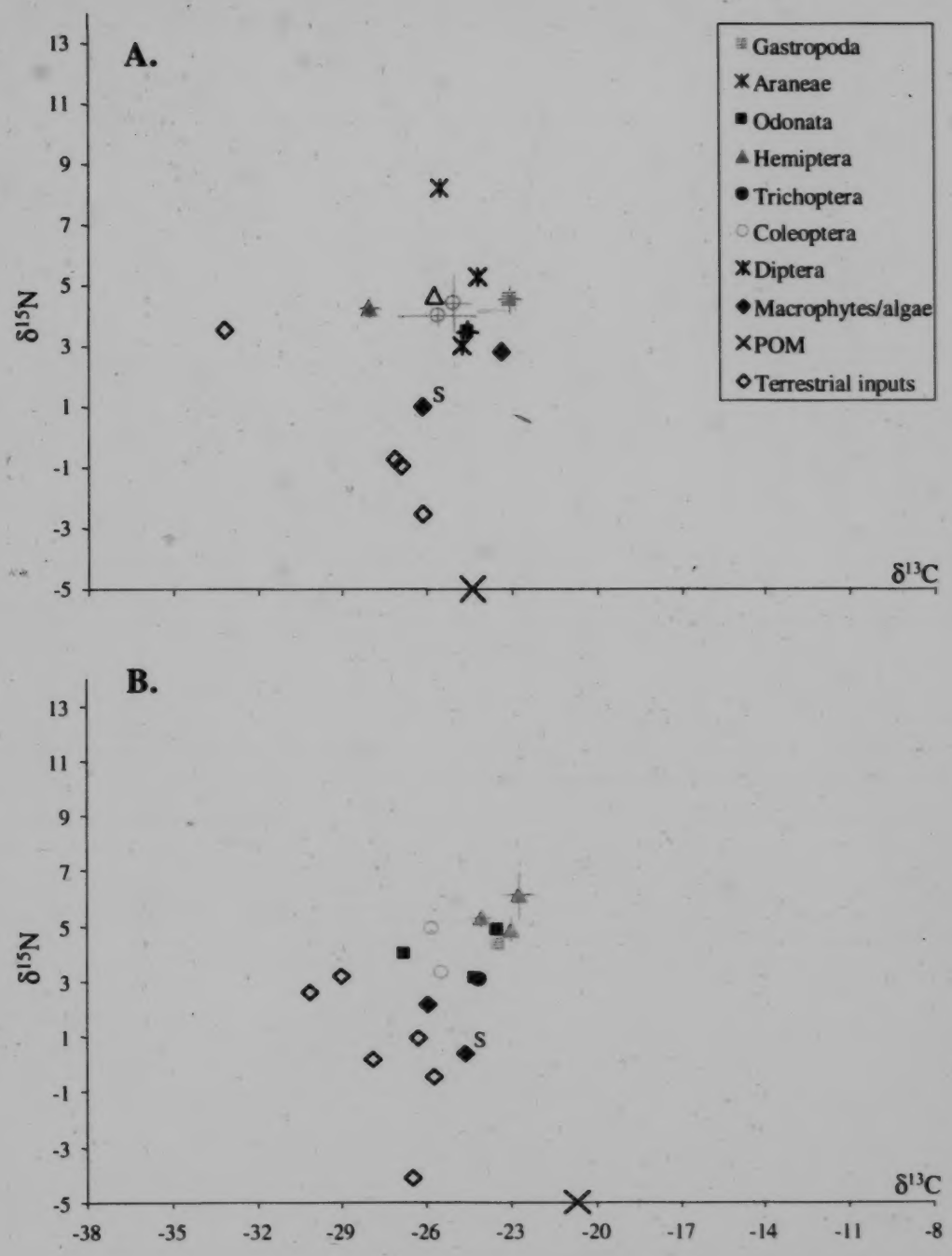

Figure 4-4. Scatter plot of $\delta^{15}N$ and $\delta^{13}C$ signatures (‰) of common taxa collected in 1999 from the temporal pond for (A) June, and (B) August. When possible, mean (±SE) values are plotted. Values and organisms listed in Appendix D, E, and F. S denotes pond sediment/benthic algae.

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Chapter V. GENERAL DISCUSSION AND CONCLUSIONS

The Whooping Crane is one of the most famous endangered species in North America, nesting only in the wetland marshes of Wood Buffalo National Park (WBNP). Although conservation efforts have increased population numbers from 16 individuals in 1941 to nearly 200 today (B. Johns, Canadian Wildlife Service, *pers. comm.*), both the low numbers and the single nesting area leave the species vulnerable. In 1994, the Whooping Crane Recovery Team published a revised plan stating that a better understanding of whooper nesting habitat was required to facilitate the identification of suitable sites for potential range expansion within WBNP and to select nesting areas appropriate for re-introductions elsewhere in North America (Edwards et al. 1994). My study, complementing an ongoing study on crane foraging and reproductive success (D. Bergeson, Parks Canada & University of Alberta, *pers. comm.*), investigated the fish-invertebrate-pond relationships within ponds used by Whooping Crane for breeding and raising young.

Since the nesting area wetlands of Wood Buffalo National Park are themselves rare and sensitive, as is the endangered species that uses them, some aspects of conventional methods commonly used to study aquatic food webs were not acceptable. Stable isotope analysis (SIA) was therefore selected as an additional, complementary method to investigate the aquatic community within the Whooping Crane breeding grounds. However, the usefulness of SIA depends on the consistency and suitability of the analytical techniques employed (Gannes et al. 1997).

Lipid extraction, a common stable-carbon isotope preparation technique, was examined to determine the unintended effects it had on stable isotope signatures of nitrogen. The experiment revealed enrichments of $\delta^{15}N$ signatures in dorsal white muscle tissue and whole fish samples, the extent of which could affect interpretation of food web studies. Therefore, the results obtained from this experiment, as well as those from other studies on preparation techniques (Bunn et al. 1995, Bosely and Wainright 1999, Pinnegar and Polunin 1999), were incorporated into the use of stable isotopes in describing the structure of aquatic communities within the wetlands of WBNP.

Specifically, acid washing and lipid extraction were not performed during the preparation of samples for SIA in Chapter IV.

The food webs of ponds within the Whooping Crane nesting area are clearly affected by both biotic and abiotic factors. Invertebrate community composition differed along gradients of area, depth, pH, conductivity, and total phosphorus. However, biotic factors, particularly the presence or absence of fish, were also important in structuring these food webs, similar to previous freshwater food web studies comparing fishless habitats to those containing fish (Macan 1977, Thorp 1986, McNicol and Wayland 1992, Hanson and Riggs 1995, Zimmer et al. 2000). Ponds containing fish were generally larger and deeper, illustrating the interaction of biotic and abiotic factors that affected overall community structure. Fishless ponds were typically more isolated, likely less affected by spring floods, and therefore less accessible to fish for colonisation. Furthermore, fishless ponds displayed a dichotomy in invertebrate community composition, defined by biotic (e.g., beetles vs. dragonflies as top predators) but correlated with abiotic (e.g., total phosphorus and conductivity) factors. Overall, the interaction of biotic (predation and competition) and abiotic factors results in three distinct aquatic communities within WBNP ponds.

The isotopic investigation of the nesting area ponds within WBNP provided information unavailable using conventional techniques. SIA was used to describe sources of primary productivity within the food webs and the trophic position of organisms. SIA also revealed distinct patterns between food webs containing and lacking fish that were likely the result of different predatory pressures and possibly altered carbon sources. SIA suggested that whoopers and fish, when present, are top predators within the diatom pond food webs, with aquatic macro-invertebrates serving as important prey for both; such information should greatly contribute to the on-going study of whoopers by Parks Canada.

The identification of community patterns and feeding interactions within the nesting area ponds of Wood Buffalo National Park has provided valuable insight into this unique wetland complex. Findings from this study will provide valuable ecological information on a virtually unknown wetland system that will aid scientists in their efforts to save the Whooping Crane.

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APPENDICES

Appendix A. Regression formula for standard body part measurements (SBM) used to estimate biomass of invertebrate food items for fish stomach content analysis.

Prey Category	Regression[e]	SBM[f]
Gastropoda[d]	N/A	
Hirudinea[d]	N/A	
Hydrachindia[d]	N/A	
Cladocera[c]	$WT = 0.00898*SBN^{3.93}$	Total length, excluding spines
Copepoda[c]	$WT = 1/3(2.20*10^{-8}*SBM+0.125*SBM^{4.4}+7.9*10^{-7}*SBM^{2.33}$	Total length, excluding spines
Ostracoda[c]	Mean = 0.012mg (SD = 0.0061, n = 5)	
Amphipoda[a]	WT = -0.54+1.153*SBM	Total length
Ephemeroptera[b]	lnW = -5.021+2.88lnSBM (r = 0.94)	Total length
Corixidae[b]	lnW = -3.461+2.40lnSBM (r= 0.93)	Total length 10mm[g]
Trichoptera[b]	lnW = -6.266+3.12lnSBM (r = 0.83)	Total length
Coleoptera[b]	lnW = -1.878+2.18lnSBM (r = 0.94)	Total length 8mm[f]
Diptera[c] (adults and larvae)	$WT = 0.839*SBM^{2.058}$ ($r^2 = 0.56$, n = 11)	Total length 8mm
Chironomidae[c]	$WT = 0.512*HL^{2.058}$ ($r^2 = 0.613$, n = 45)	Head length (HL)
Ceratopogonidae[b]	lnW = -5.221+2.43lnSBM (r = 0.96)	Total length 10mm[g]
Detritus	N/A	
Eggs[d]	N/A	

[a] (University of Alberta - "TROLS" unpublished data)
[b] (Smock 1980)
[c] (Litvak and Hansell 1990)
[d] volume calculated
[e] Dry mass (WT) in mg
[f] Standard body measure (mm)
[g] SBM from Clifford (1991)

Appendix B. List of invertebrates found in the 36 ponds within the Whooping Crane nesting area. Names in bold indicate invertebrates and taxonomic level used in ordination. The codes refer to those used for figures in Chapter III.

Class	Subclass	Order	Suborder	Family	Subfamily	Genus Species	Codes
DEMOSPONGIAE				Spongillidae			
	Nematoda						
CLITELLATA	Hirudinea						
GASTROPODA	Pulmonata			**Planorbidae**			Plan
				Lymnaeidae			Lymn
ARACHNIDA	Acari	Hydrachnidia					Arac
		Araneae		Pisauridae		*Dolomedes trition*	
CRUSTACEA	Branchiopoda	**Cladocera**					Clad
	Copepoda						Cope
	Ostracoda						Ostr
	Malacostraca	Amphipoda				***Hyalella azteca***	Hyal
INSECTA		Ephemeroptera					Ephe
				Caenidae		***Caenis* sp.**	Caen
				Siphlonuridae			
		Odonata	Anisoptera				Anis
				Aeshnidae		***Aeshna* sp.**	Aesh
				Libellulidae		***Leucorrhinia* sp.**	Leuc
						***Libellula* sp.**	Libe
						Pachydiplax sp.	
						Sympetrum sp.	
			Zygoptera				Zygo
				Lestidae		***Lestes* sp.**	Lest
				Coenagrionidae		***Enallagma* sp.**	Enal
		Hemiptera		Belestomatidae		***Lethocerus* sp.**	Leth
				Corixidae			Cori
				Gerridae		***Gerris* sp.**	Gerr
						***Limnoporus* sp.**	Limn

Appendix B. (continued)

Class	Subclass	Order	Suborder	Family	Subfamily	Genus Species	Codes
INSECTA cont.		Hemiptera cont.		Notonectidae		***Notonecta* sp.**	Noto
		Trichoptera					Tric
				Phryganeidae		*Phryganea* sp.	
						Ptilostomis sp.	
				Limnephilidae			
				Philoptomidae		*Dolophilodes* sp.	
				Leptoceridae		*Oecetis* sp.	
				Brachycentridae			
		Coleoptera					Cole
				Dytiscidae	Dytiscinae	***Graphoderus* sp.**	Grap
						***Acilius* sp.**	Acil
						***Dytiscus* sp.**	Dyti
					Colymbetinae	*Agabus* sp.	Coly
						Carrhydrus sp.	
						Colymbetes sp.	
						Neoscutopterus sp.	
					Hydroporinae	*Hygrotus* sp.	
				Hydrohilidae		*Enochrus* sp.	
				Gyrinidae		*Gyrinus* sp.	
				Scirtidae			
		Diptera					Dipt
			Nematocera	**Chironomidae**			Chir
				Chaoboridae			
				Ceratopogonidae			
				Psychodidae		*Pericoma* sp.	
			Brachycera	Syrphidae		*Eristalis* sp.	
				Ephydridae			
				Stratiomyidae			

Appendix C. Percent Frequency of Occurrence, number, mass, and Relative Importance (RI) of prey taxa in the diet of STBK, DACE, and FTHD in the wetland complex of Wood Buffalo National Park. *indicates % total volume (see Chapter III; Methods & Materials). Diptera - P refers to Diptera, other than Ceratopogonidae and Chironomidae, found in the pupal stage of development.

Fish	Prey Taxon	% Frequency Occurrence	% Number	% Mass	% RI
STBK	Copepoda	13.3	18.9	1.2	7.1
	Cladocera	53.3	30.4	0.3	17.9
	Coleoptera	6.7	0.2	0.9	1.6
	Ceratopogonidae	3.3	0.1	1.2	1.0
	Chironomidae	83.3	28.3	11.6	26.3
	Diptera - P	30.0	1.8	10.3	9.0
	Ephemeroptera	76.7	6.7	72.3	33.2
	Hydrachnidia	3.3	0.1	0.0	0.7
	Ostracoda	60.0	12.8	1.3	15.8
	Trichoptera	13.3	0.5	1.0	3.2
	Eggs	3.3	0.2	0.0	0.8
	Detritus	6.7	-	1.0*	7.9
DACE	Copepoda	6.7	1.2	0.0	1.7
	Cladocera	6.7	6.1	0.0	2.7
	Coleoptera	3.3	0.6	0.5	0.9
	Ceratopogonidae	6.7	1.2	1.1	1.9
	Chironomidae	33.3	21.3	0.4	11.8
	Diptera - A	16.7	4.3	14.2	7.5
	Diptera - P	26.7	9.8	6.4	9.1
	Ephemeroptera	53.3	30.5	64.4	31.6
	Gastropoda	3.3	7.3	0.0	2.3
	Hydrachnidia	6.7	1.2	0.0	1.7
	Ostracoda	10.0	6.1	0.0	3.4
	Odonata	23.3	8.5	11.4	9.2
	Hemiptera	10.0	1.8	1.5	2.8
	Detritus	36.7	-	7.3*	45.5
FTHD	Coleoptera	25.0	1.1	5.3	6.7
	Chironomidae	75.0	90.1	16.1	38.7
	Ephemeroptera	50.0	8.8	78.6	29.3
	Detritus	25.0	-	20.0*	46.6

Appendix D. Principle Component Analysis original data matrix for the output of Figure3- 4.

Pond	Plan	Lymn	Arac	Clad	Cope	Ostr	Hyal	Caen	Aesh	Leuc	Libe	Enal	Leth	Cori	Gerr	Limn	Noto	Tri	Grap	Acil	Dyti	Coly	Chir
1A	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1	0	0	0	1	0	1
1B	1	1	1	0	1	1	0	1	1	1	1	1	0	1	0	0	0	1	0	0	0	0	1
2A	1	1	1	1	0	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1
2B	1	1	1	1	1	1	0	0	1	1	1	0	1	1	0	0	1	0	0	0	0	0	1
3A-98	1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1
3B	1	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	1	1
4A	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	0	1	0	0	0	1	1	1
4B	1	1	1	1	1	1	1	0	1	1	0	1	0	0	0	0	1	0	0	0	0	1	1
5A	1	1	1	0	0	0	0	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0	1
5B	1	1	1	1	1	1	0	1	1	0	1	1	0	1	0	0	1	0	1	0	0	1	1
6A	1	1	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
6B	0	1	1	0	1	0	0	1	1	0	1	0	0	1	0	0	1	0	0	1	0	0	1
7A	1	1	1	0	1	0	1	1	1	0	1	0	1	0	0	0	1	0	1	0	0	0	1
7B	1	0	1	0	1	0	0	1	1	0	1	0	1	1	0	0	1	0	0	1	1	0	1
8A	0	1	1	0	1	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1
8B	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
3C	0	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
3A-99	1	1	1	0	1	0	0	0	1	1	1	0	0	0	1	0	1	0	0	1	0	1	0
9A	1	1	1	1	1	1	0	0	1	1	1	0	1	1	1	0	1	1	0	0	0	0	0
9B	0	1	1	1	1	1	0	1	1	1	0	1	0	0	1	1	1	1	1	0	0	0	0
10A	1	1	1	1	1	0	0	1	1	1	1	0	1	1	1	1	1	1	1	0	0	0	1
10B	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	0	0	0	1	1
11A	0	1	1	1	0	0	1	0	1	1	1	0	1	0	0	0	1	0	1	0	1	0	0
11B	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	1	0
12A	1	1	1	0	0	1	1	0	1	0	0	0	1	0	1	0	0	1	0	0	0	0	1
12B	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	0
13A	1	1	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1
13B	0	0	1	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0
14A	0	1	1	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	1	0	0	1	1
14B	1	1	1	0	0	0	1	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	1
15A	1	1	1	0	1	0	1	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0

Appendix D. (continued)

Pond	Plan	Lymn	Arac	Clad	Cope	Ostr	Hyal	Caen	Aesh	Leuc	Libe	Enal	Leth	Cori	Gerr	Limn	Noto	Tri	Grap	Acil	Dyti	Coly	Chir
15B	1	1	1	0	1	0	0	1	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0
16A	0	1	1	1	1	0	0	0	1	0	1	0	1	0	1	1	1	0	1	0	0	1	0
16B	0	0	1	1	1	0	1	0	0	0	1	0	0	0	1	0	1	1	1	0	0	0	0
17A	0	1	1	1	0	1	1	1	1	1	1	0	1	0	1	0	1	1	0	0	1	0	1
17B	0	1	1	1	0	1	0	0	1	0	0	0	0	0	1	1	0	0	1	0	1	1	0

Appendix E. Means (±SE) of $\delta^{13}C$ and $\delta^{15}N$ signatures (‰) of vertebrates from nesting area ponds, Wood Buffalo National Park.

Territory (Pond)	Year	Location	Organism	n	$\delta^{13}C$ mean (±SE)	$\delta^{15}N$ mean (±SE)
1	1998	Preble Creek	Whooping Crane	1	-27.7	7.6
1A			Brook stickleback	9	-31.0(±0.12)	7.5(±0.06)
3	1998	Klewi River	Whooping Crane	3	-17.1(±1.86)	11.6(±1.32)
3A-98			Brook stickleback	10	-27.4(±0.21)	6.3(±0.11)
			Dace*	8	-27.8(±0.25)	5.3(±0.08)
3	1999	Klewi River	Whooping Crane	1	-19.2	13.3
3A-99			Brook stickleback	4	-26.4(±0.20)	6.3(±0.19)
			Dace*	1	-27.8	5.1
3C			Wood Frog	1	-23.77	5.7
			tadpoles	2	-22.5(±0.24)	6.8(±0.01)
8	1998	Sass River	Whooping Crane	4	-27.3(±1.54)	7.0(±0.24)
13	1999	Sass River	Whooping Crane	2	-24.9(±0.50)	8.8(±0.33)
13A			Brook stickleback	5	-30.1(±0.83)	7.9(±0.25)
			Dace*	10	-30.1(±0.50)	7.2(±0.13)
			Fathead minnow	5	-32.7(±0.64)	8.0(±0.58)
15	1998	Klewi River	Whooping Crane	1	-15.8	11.6
16	1999	Klewi River	Whooping Crane	2	-21.8(±0.14)	11.4(±0.28)

*composite of northern redbelly, finescale, and pearl dace, plus hybrids.

Appendix F. Means (±SE) of $\delta^{13}C$ and $\delta^{15}N$ signatures (‰) of invertebrates from nesting area and temporal ponds.

Pond	Year		Organism	n	$\delta^{13}C$ mean (±SE)	$\delta^{15}N$ mean (±SE)
1A	1998	Gastropoda	Lymnaeidae	1	-31.6	2.6
		Amphipoda	*Hyalella azteca*	1	-34.4	-0.2
		Odonata	Anisoptera - *Aeshna* sp.	4	-32.0(±0.33)	2.6(±0.45)
		Odonata	Anisotpera - *Libellula* sp.	1	-32.2	1.8
		Odonata	Anisoptera - *Leucorrhinia* sp.	1	-32.6	1.3
		Hemiptera	Belestomatidae	1	-31.8	4.8
		Hemiptera	Notonectidae	3	-30.9(±0.21)	3.0(±0.15)
		Hemiptera	Corixidae	3	-30.5(±0.40)	1.1(±0.66)
		Coleoptera	*Dytiscus* sp.	1	-32.6	3.5
1B	1998	Gastropoda	Lymnaeidae	4	-30.4(±0.82)	2.1(±0.20)
		Crustacea	Copepoda - Calanoida	1	-31.8	4.1
		Odonata	Zygoptera - *Enallagma* sp.	2	-29.5(±0.24)	3.5(±0.48)
		Odonata	Anisoptera - *Aeshna* sp.	4	-29.9(±0.13)	2.6(±0.13)
		Odonata	Anisotpera - *Libellula* sp.	2	-29.5(±0.01)	1.3(±0.26)
		Hemiptera	Corixidae	1	-28.0	1.2
		Coleoptera	*Hydaticus* sp.	1	-33.8	2.8
		Diptera	Chaoboridae	1	-27.9	4.8
3A-98	1998	Ephemeroptera	Caenidae	1	-30.5	0.2
		Odonata	Anisoptera - *Aeshna* sp.	2	-27.9(±0.35)	1.3(±0.35)
		Hemiptera	Notonectidae	3	-27.3(±0.10)	3.5(±0.23)
		Hemiptera	Gerridae	2	-27.6(±0.21)	3.3(±0.39)
		Diptera	Chironomidae	1	-28.7	1.8
3B	1998	Gastropoda	Lymnaeidae	3	-20.5(±0.26)	5.0(±0.27)
		Odonata	Anisoptera - *Libellula* sp.	1	-21.1	4.8
		Hemiptera	Notonectidae	1	-23.0	4.2
		Hemiptera	Corixidae	1	-21.7	4.1
		Coleoptera	*Graphoderus* sp.	1	-26.9	5.8
		Coleoptera	*Enochrus* sp.	1	-19.7	4.7
		Diptera	Chironomidae	1	-22.2	4.4
3A-99	1999	Gastropoda	Lymnaeidae	2	-25.9(±0.16)	2.7(±0.11)
		Gastropoda	Planorbidae	1	-27.2	3.5
		Hirudinea	Glossiphoniidae	1	-26.7	4.8
		Araneae	*Dolomedes trition*	1	-27.7	6.7
		Odontata	Anisoptera - *Aeshna* sp.	4	-28.3(±0.24)	2.8(±0.18)
		Odontata	Anisotpera - *Libellula* sp.	4	-22.6(±0.30)	6.8(±0.17)
		Odontata	Anisoptera - *Leucorrhinia* sp.	1	-28.8	2.2
		Hemiptera	Notonectidae	4	-27.2(±0.08)	4.1(±0.25)
		Hemiptera	Gerridae	2	-27.3(±0.37)	4.3(±0.77)
		Trichoptera	Limnephilidae	1	-31.0	-1.7
		Trichotpera	Phyganeidae	1	-28.3	3.3
		Coleoptera	*Acilius* sp.	1	-30.2	4.7
		Coleoptera	Colymbetinae	2	-28.5(±0.48)	3.3(±0.83)

Appendix F. (continued)

Pond	Year		Organism	n	$\delta^{13}C$ mean (±SE)	$\delta^{15}N$ mean (±SE)
3C	1999	Gastropoda	Lymnaeidae	3	-20.7(±0.06)	7.6(±0.27)
13A	1999	Ephemeroptera	Siphlonuridae	1	-33.5	1.4
		Odonata	Anisoptera - *Aeshna* sp.	2	-31.1(±0.42)	4.3(±0.04)
		Odonata	Anisotpera - *Libellula* sp.	2	-31.0(±0.21)	2.7(±0.18)
		Hemiptera	Gerridae	3	-29.4(±0.49)	4.1(±0.30)
13B	1999	Gastropoda	Lymnaeidae	3	-24.9(±0.38)	7.0(±0.17)
		Ephemeroptera		1	-27.0	0.5
		Odonata	Zygoptera - Lestes sp.	1	-26.0	9.4
		Odonata	Anisoptera - *Libellula* sp.	3	-25.9(±0.11)	7.4(±0.33)
		Hemiptera	Corixidae	1	-24.6	6.8
		Hemiptera	Gerridae	3	-26.5(±0.61)	5.5(±0.51)
		Trichoptera	Lymnephilidae	1	-26.1	7.4
		Coleoptera	Graphoderus	3	-26.3(±0.42)	7.3(±0.99)
Temporal Pond						
	June	Gastropoda	Lymnaeidae	2	-23.1(±0.45)	4.5(±0.47)
		Araneae	*Dolomedes trition*	1	-25.6	8.2
		Odonata	*Libellula* sp.	4	-24.5(±0.37)	3.5(±0.31)
		Hemiptera	Gerridae	6	-28.0(±0.38)	4.3(±0.33)
		Coleoptera	*Graphoderus* sp.	2	-25.6(±1.37)	4.0(±0.29)
		Coleoptera	Colymbetinae	2	-25.0(±0.60)	4.0(±0.95)
		Diptera	Chironomidae	1	-24.8	3.0
		Diptera	*Eristalis* sp.	1	-24.2	5.3
	August	Gastropoda	Lymnaeidae	4	-23.5(±0.27)	4.3(±0.05)
		Odonata	Zygoptera - *Lestes* sp.	1	-26.8	4.0
		Odonata	Anisoptera - *Aeshna* sp.	1	-23.5	4.8
		Odonata	Anisoptera - *Libellula* sp.	4	-24.2(±0.26)	3.1(±0.15)
		Hemiptera	Belestomatidae	2	-23.0(±0.06)	4.9(±0.41)
		Hemiptera	Notonectidae	4	-22.7(±0.57)	6.1(±0.85)
		Hemiptera	Gerridae	4	-24.1(±0.39)	5.3(±0.29)
		Trichoptera	Limnephilidae	1	-24.1	3.1
		Coleoptera	*Acilius* sp.	1	-25.8	5.0
		Coleoptera	*Graphoderus* sp.	4	-25.5(±0.21)	3.3(±0.08)

Appendix G. Means of $\delta^{13}C$ and $\delta^{15}N$ signatures (‰) of primary producers, aquatic and terrestrial, from nesting area and temporal ponds, Wood Buffalo National Park.

Pond	Year/ Month	Organism	$\delta^{13}C$ mean	$\delta^{15}N$ mean
1A	1998	Bladderwort	-31.5	4.0
		Cattail	-27.7	2.1
		Bulrish	-25.9	3.9
		Sedge	-24.3	3.6
		Pond Sediment/Benthic algae	-30.0	0.4
		D. birch	-27.2	-1.7
		Moss	-33.8	2.2
		Labrador Tea	-27.5	-4.3
		Salix sp. #1	-27.5	0.2
		Vaccinium sp.	-27.8	-4.2
		Soil	-27.4	-0.4
1B	1998	Bladderwort	-31.4	2.3
		Cattail	-27.3	2.6
		Sedge	-27.8	1.4
		Pond Sediment/Benthic algae	-29.0	-0.2
		Moss	-29.2	3.4
		Labrodor Tea	-26.9	-3.1
		Salix sp.	-29.2	0.8
		Vaccinium sp.	-29.0	-4.4
		Tamarack	-29.7	-2.8
		Dwarf Birch	-27.5	-1.4
		Soil	-27.8	-0.2
3A-98	1998	Bladderwort	-28.4	2.4
		Bulrush	-27.0	1.6
		Pond Sediment/Benthic algae	-28.1	-0.1
		Particulate Organic Matter	-36.6	-
		Moss	-28.5	-0.1
		Calamagrostis inexpansa	-27.3	1.4
		Labrador Tea	-27.9	-0.7
		Salix sp. #1	-28.1	-0.6
		Salix sp. #2	-28.5	-0.9
		Potentilla sp. #1	-27.3	-0.5
		Potentilla sp. #2	-26.8	4.6
		Dwarf Birch	-27.8	-0.9
		Tamarack	-29.6	-2.2
		Soil	-26.8	-0.3
3B	1998	Pond Sediment/Benthic algae	-22.8	2.3
		Particulate Organic Matter	-18.8	-
		Labrador Tea	-25.8	-2.9
		Potentilla sp. #2	-26.7	0.7
		Salix sp. #1	-27.2	0.8

Appendix G. (continued)

Pond	Year/ Month	Organism	$\delta^{13}C$ mean	$\delta^{15}N$ mean
		Dwarf Birch	-26.7	-1.3
		Soil	-27.1	-0.3
3A-99	1999	Bladderwort	-28.6	3.1
		Bulrush	-26.3	1.6
		Pond Sediment/Benthic algae	-28.2	-0.6
		Particulate Organic Matter	-26.6	-
		Dissolved Inorganic Carbon	-6.1	-
		Labrador Tea	-25.8	-1.5
		Potentilla sp. #2	-25.8	-1.2
		Salix sp. #1	-25.9	-0.3
		Dwarf Birch	-26.4	-0.1
		Tamarack	-27.3	-1.0
		Spruce	-26.6	-2.7
		Soil	-26.1	0.2
3C	1999	Sedge	-26.1	4.2
		Pond Sediment/Benthic algae	-22.6	4.6
		Dissolved Inorganic Carbon	-9.5	-
		Labrador Tea	-25.7	-0.9
		Potentilla sp. #2	-25.6	1.2
		Salix sp. #1	-25.9	-0.1
		Dwarf Birch	-26.2	0.6
		Soil	-26.7	0.1
13A	1999	Bladderwort	-31.7	3.5
		Bulrush	-27.7	-1.2
		Sedge	-26.8	1.8
		Pond Sediment/Benthic algae	-30.3	1.2
		Dissolved Inorganic Carbon	-8.0	-
		Potentilla sp. #2	-26.7	-0.1
		Salix sp. #1	-26.9	0.2
		Soil	-26.7	1.1
13B	1999	Bulrush	-25.3	4.7
		Sedge	-25.7	0.3
		Pond Sediment/Benthic algae	-24.0	4.8
		Particulate Organic Matter	-18.6	-
		Dissolved Inorganic Carbon	-8.7	-
		Labrador Tea	-25.7	-0.3
		Potentilla sp. #2	-26.3	0.3
		Salix sp. #1	-27.5	0.3
		Dwarf Birch	-26.6	0.2
		Soil	-26.7	-1.3
Temporal Pond	June	*Chara* sp.	-23.4	2.8
		Pond Sediment/Benthic algae	-26.1	1.0
		Particulate Organic Matter	-24.4	-

Appendix G. (continued)

Pond	Year/ Month	Organism	$\delta^{13}C$ mean	$\delta^{15}N$ mean
		Dissolved Inorganic Carbon	-7.5	-
		Moss	-33.2	3.5
		Labrador Tea	-26.2	-2.5
		Salix sp. #1	-27.2	-0.8
		Dwarf Birch	-27.0	-0.9
		Spruce	-27.1	-6.0
	August	Sedge	-26.0	2.2
		Scirpus sp.	-29.1	3.2
		Pond Sediment/Benthic algae	-24.6	0.4
		* Particulate Organic Matter	-20.7	-
		Dissolved Inorganic Carbon	-8.2	-
		Moss	-30.2	2.6
		Labrador Tea	-25.7	-0.5
		Dwarf Birch	-27.9	0.2
		Spruce	-26.5	-4.1
		Soil	-26.3	1.0

* collected July 17, 1999